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# NOTICE of CHANGE dated 28/11/2023

## IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

# «BCR-ABL P190 ELITe MGB® Kit»
## Ref. RTSG07PLD190

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Updated calibration curve validity (60 days)*
- *Updated transport and storage conditions for primary sample*

The product can be used with the previous versions of the IFU as well.

*Composition, use and performance of the product remain unchanged*

## PLEASE NOTE

| | |
|---|---|
| | **LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT** |
| | **THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT** |
| | **CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT** |
| | **LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT** |
| | **A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT** |
| | **DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT** |

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EMPOWERING IVD

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# BCR-ABL P190 ELITe MGB® Kit

reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTSG07PLD190 | CE | IVD | -20°C

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## INTENDED USE

The **«BCR-ABL P190 ELITe MGB® Kit»** product is a qualitative and quantitative, reverse transcription and amplification of nucleic acids assay for the **detection of the mRNA of the BCR-ABL rearrangement, t(9;22) translocation, *Philadelphia* chromosome, variant P190 (P190)** and for the **quantification of the mRNA of P190 compared with the mRNA of the gene codifying the kinase protein Abelson (ABL)** in total RNA samples extracted from lymphomonocyte suspensions and leukocyte suspensions from clinical samples of peripheral blood or bone marrow.

The product is intended for use, alongside patient clinical data and other laboratory tests, as an help in the diagnosis and monitoring of cases of chronic myeloid leukaemia (CML), acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) positive for the P190 marker.





## ASSAY PRINCIPLE

The assay consists of a reverse transcription and a real time amplification reaction (one-step method) with a programmable thermostat provided with a fluorescence detection optical system (real time amplification thermal cycler).

For each extracted RNA sample, the assay involves **a duplicate specific reaction for a P190 mRNA region** (target) and **a duplicate specific reaction for an ABL mRNA region** (control).

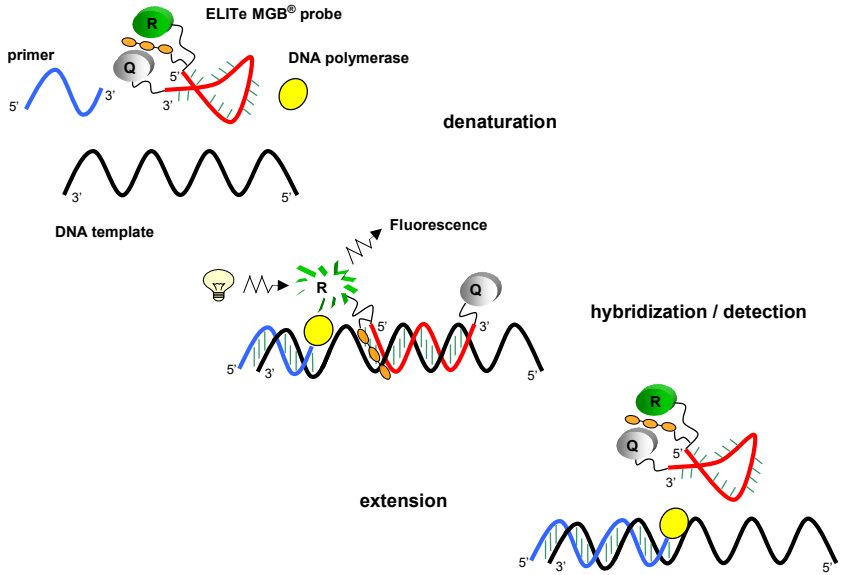
The P190 cDNA specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the P190 cDNA amplification reaction.

The ABL cDNA specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of ABL cDNA amplification reaction.

As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data determines the presence and the titre of P190 and ABL mRNA in the starting sample.

The assay is validated for use in association with the systems described in this user manual.

The following picture shows the mechanism of activation and fluorescence emission of the ELITe MGB® technology probe. Note that the probe is not hydrolyzed during the amplification cycles.



## PRODUCT DESCRIPTION

The **«BCR-ABL P190 ELITe MGB® Kit»** product provides the following components:

- **P190 PreMix**

A mixture of oligonucleotides, specific for P190 reverse transcription and real time amplification, in a stabilized solution, **aliquoted into a test tube** (PURPLE cap), containing **270 µL** of solution, sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

Primer oligonucleotides and the specific probe for P190 (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non-fluorescent molecule), which are specific for a region of the mRNA generated by **BCR-ABL rearrangement variant P190 (e1a2)**.

The reaction mixture provides AP593 fluorophore, used instead of ROX or CY5, as passive reference for fluorescence normalisation.

- **ABL PreMix**

A mixture of oligonucleotides, specific for ABL reverse transcription and real time amplification, in a stabilized solution, **aliquoted into a test tube** (NEUTRAL cap), containing **270 µL** of solution, sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

Primer oligonucleotides and the specific probe for ABL (stabilized by MGB® group, labelled by FAM fluorophore and quenched by a non fluorescent molecule) which are specific for a region of the mRNA of the human gene encoding **ABL (exons a2a3)**.

The reaction mixture provides AP593 fluorophore, used instead of ROX or CY5, as passive reference for fluorescence normalisation.

- **PCR MasterMix**

A mixture of optimized and stabilized reagents for reverse transcription and real time amplification **aliquoted into 2 test tubes** (NEUTRAL cap). Each tube contains **820 µL** of solution, which is sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

The reaction mixture provides the buffer, magnesium chloride, the nucleotide triphosphates and the hot start Taq DNA polymerase enzyme.

- **RT EnzymeMix**

A mixture of optimized and stabilized reagents for reverse transcription, **aliquoted into 2 test tubes** (cap with BLACK insert). Each tube contains **20 µL** of solution, sufficient for at least **36 tests** in association with **«ELITe InGenius®»** and **50 tests** in association with other systems.

The reaction mixture provides the reverse transcriptase enzyme.

The product enables **18 duplicate determinations for the mRNA of P190** and **18 duplicate determinations for the mRNA of ABL in association with ELITe InGenius**, including standards and controls.

The product enables **25 duplicate determinations for the mRNA of P190** and **25 duplicate determinations for the mRNA of ABL in association with** 7300 Real Time PCR System, 7500 Fast Dx Real-Time PCR Instrument and 7900 Real-Time PCR System, including standards and controls, i.e. a maximum number of **19 clinical samples** in one session (under optimal conditions of use).



**BCR-ABL P190 ELITe MGB® Kit**
reagents for RNA reverse transcription and cDNA Real Time amplification

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## MATERIALS PROVIDED IN THE PRODUCT

| Component | Description | Quantity | Hazard classification |
|---|---|---|---|
| **P190 PreMix** | Primer/probe oligonucleotides mixture PURPLE cap | **1 x 270 µL** | - |
| **ABL PreMix** | Primer/probe oligonucleotides mixture NEUTRAL cap | **1 x 270 µL** | - |
| **PCR MasterMix** | mixture of reagents for reverse transcription and real time amplification NEUTRAL cap | **2 x 820 µL** | - |
| **RT EnzymeMix** | Reverse transcriptase cap with BLACK insert | **2 x 20 µL** | - |

## MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Sterile micropipettes and tips with aerosol filter or positive displacement (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.
- Sarstedt 2.0 mL skirted tube with screw-cap (Sarstedt Ref. 72.694.005).
- Polypropylene 1.5 mL microtubes for molecular biology.
- Programmable thermostat with optical fluorescence detection system 7300 Real Time PCR System, 7500 Fast Dx Real-Time PCR Instrument or 7900 Real-Time PCR System calibrated following manufacturer's instructions.

## OTHER PRODUCTS REQUIRED

The reagents for the extraction of RNA from samples, amplification microplates and known-quantity DNA standards **are not** included in this product.

For automatic sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) the following generic products are required: the extraction cartridges **«ELITe InGenius® SP RNA»** (ELITechGroup S.p.A., ref. INT034SPRNA), the **«ELITe InGenius DNase I»** (ELITechGroup S.p.A. INT034DNASE), the **«Dnase Tube Adapter Kit»** (ref. G6431-000), the consumables for extraction and amplification of nucleic acids from biological samples **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S).

For automatic RNA extraction, amplification and interpretation of sample analysis, the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:

for the calibrators **«BCR-ABL P190 ELITe_STD_P190»** and **«BCR-ABL P190 ELITe_STD_ABL»**,

for the positive control of amplification **«BCR-ABL P190 ELITe_PC»**,

for negative control of amplification **«BCR-ABL P190 ELITe_NC»**,

for samples analysis **«BCR-ABL P190 ELITe_PBL_200_100».**

For RNA extraction from samples to be analysed, use a laboratory validated product, such as the **«Maxwell® CSC»** (Promega, code AS6000) automatic extraction system with **Maxwell® CSC RNA Blood Kit** reagents (Promega, code AS1410) or other equivalent products.

When 7300 Real-Time PCR System is used, it is required the use of generic product: **«MicroAmp™ Optical 96-Well Reaction Plate»** (Life Technologies, ref. N8010560), microplates with 0.2 mL wells and adhesive sealing sheets for real time amplification.

When 7500 Fast Dx Real-Time PCR Instrument is used, it is required the use of generic product: **«MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL»** (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

For detection and quantification of P190 mRNA and ABL mRNA, the product **«BCR-ABL P190 - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTRG07PLD190), positive control of plasmid DNA, is required.

For the detection and quantification of P190 mRNA and ABL mRNA, the product **«BCR-ABL P190 ELITe Standard»** (ELITechGroup S.p.A., code STDG07PLD190), five dilutions of known-quantity plasmid DNA to obtain P190 and ABL standard curves, is required.

For blood pre-treatment, use a laboratory validated generic product, such as the Cell Lysis Solution (Promega, Ref. A7933), RNA Lysis Buffer (Promega, Ref. Z3051) and Thioglycerol (Promega, Ref. A208B-C) or equivalent reagents (such as Solution A (Promega, Ref. MC130A), Solution B (Promega, Ref. MC131A) and Thioglycerol (Promega, Ref. MC132A).

## WARNINGS AND PRECAUTIONS

**This product is exclusively designed for *in-vitro* use.**

**General warnings and precautions**

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Carefully read all instructions provided in the product before running the assay.
While running the assay, follow the instructions provided in the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not mix reagents from different batches.
Do not use reagents from other manufacturers.

**Warnings and precautions for molecular biology**

Molecular biology procedures, such as nucleic acid extraction, reverse transcription, amplification and detection, require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained within the samples or sample contamination by amplification products.



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When amplification session is manually setup, It is necessary to have available separate areas for the extraction / preparation of amplification reactions and for the amplification / detection of amplification products. Never introduce an amplification product into the area designated for extraction / preparation of amplification reactions.

When amplification session is manually setup, It is necessary to have available lab coats, gloves and tools which are exclusively used for the extraction / preparation of the amplification reactions and for the amplification / detection of amplification products. Never transfer lab coats, gloves or tools from the area designated for the amplification / detection of amplification products to the area designated for the extraction / preparation of the amplification reactions.

The samples must be exclusively used for this type of analysis. Samples must be handled under a laminar airflow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNAses and RNAses, and free from DNA and RNA.

Amplification products must be handled in such a way as to reduce dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle amplification products must be exclusively used for this purpose.

**Warnings and precautions specific for the components**

- **P190 PreMix**
  The **P190 PreMix** must be stored at -20 °C in the dark.
  The **P190 PreMix** can be frozen and thawed for no more than **six times**: further freezing / thawing cycles may reduce product performance.
- **ABL PreMix**
  The **ABL PreMix** must be stored at -20 °C in the dark.
  The **ABL PreMix** can be frozen and thawed for no more than **six times**: further freezing / thawing cycles may reduce product performance.
- **PCR MasterMix**
  The **PCR MasterMix** must be stored at -20 °C.
  The **PCR MasterMix** can be frozen and thawed for no more than **six times**: further freezing / thawing cycles may reduce product performance.
- **RT EnzymeMix**
  The **RT EnzymeMix** must be stored at -20 °C.
  The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes for no more than **six times**.

## ELITe InGenius®

## SAMPLES AND CONTROLS

**Samples**

This product must be used with the following clinical samples:

**Peripheral blood collected in EDTA or sodium citrate**

The peripheral blood collected in EDTA or sodium citrate, used for lymphomonocyte and leukocyte suspensions preparation for RNA extraction, must be collected according to laboratory guidelines, transported and stored at room temperature (+21 ±5 °C) for a maximum of 24 hours.

Do not freeze peripheral blood in order to prevent degradation of RNA.

When starting with peripheral blood it is advisable to separate leukocyte according to laboratory guidelines or following indications.

Transfer 10 – 14 mL of fresh peripheral blood collected in EDTA or sodium citrate into a 15 mL tube after mixing it thoroughly by inversion. Centrifuge for 10 minutes at 3000 RCF; add 5 mL of Cell Lysis Solution (Promega, Ref. A7933) into a new 15 mL tube; with a 1 mL pipette, remove the buffy-coat obtained after centrifugation and transfer it to the 15 mL tube containing the lysis solution; aspirate and release until the cells are inside the tube and the pipette is free of material; incubate at room temperature for 10 minutes and mix by inversion (NO VORTEX) at least 3-4 times; centrifuge at 3000 RCF for 10 minutes

**Note:** the ideal amount of white cells, is represented, in 1:1 scale, in the following picture

Remove the supernatant and resuspend in 2 mL of Cell Lysis Solution by transferring it into a 2 mL tube; centrifuge again for about 2 minutes at 3000 RCF; carefully remove the supernatant (attention to remove traces of red cells above the with cells pellet) and resuspend the pellet in 200 µL of Lysis Solution (1 mL of RNA Lysis Buffer, Promega, Ref. Z3051 + 20 µL of 1-Thioglycerol, Promega, Ref. A208B-C).

**Note:** when nucleic acid extraction is carried out with the **ELITe InGenius** and with **ELITe InGenius® Software** version 1.3 (or later equivalent versions), use the extraction protocols **BCR-ABL P190 ELITe_PBL_200_100**. This protocol processes 200 µL of sample and elutes the nucleic acids in 100 µL.

**Interfering substances**

The extracted RNA must not contain heparin, haemoglobin, Ficoll®, ethanol or 2-propanol in order to prevent inhibition and the possibility of frequent invalid results.

Quantities of RNA more than 2.0 µg per reaction could inhibit the reverse transcription reaction and the real time amplification.

Quantities of human genomic DNA higher than 100 ng per reaction in the RNA extracted from the sample could inhibit the reverse transcription reaction and the real time amplification.



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## PROCEDURE

The procedure to use the **BCR-ABL P190 ELITe MGB® Kit** with the system **ELITe InGenius** consists of three steps:

- Verification of the system readiness,
- Set up of the session,
- Review and export of results.

**System readiness verification**

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the **ELITe InGenius** and select the mode "**CLOSED**",
- verify that the Calibrators (**BCR-ABL P190 Q-PCR Standard**) were run, approved and are not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Calibrators approved or valid, run them as described in the following paragraphs,
- verify that the amplification Controls (Controls, **BCR-ABL P190 Positive Control, BCR-ABL P190 Negative Control**) were run, approved and are not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Controls approved or valid, run them as described in the following paragraphs,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB® kits, **ELITe InGenius** instrument and the cited matrix.

The Assay Protocol available for sample testing with the product **BCR-ABL P190 ELITe MGB® Kit** is described in the table below.

| Assay protocol for BCR-ABL P190 ELITe MGB® kit | | | |
|---|---|---|---|
| **Name** | **Matrix** | **Report unitage** | **Characteristics** |
| **BCR-ABL P190 ELITe_PBL_200_100** | Peripheral Blood Leukocyte | %P190 | Extraction Input Volume: 200 µL<br>Extracted Elute Volume: 100 µL<br>Sonication: NO<br>Internal Control: NO<br>PCR Mix volume: 20 µL<br>Sample PCR input volume: 10 µL |

If the assay protocol of interest is not in the system, contact your local ELITechGroup Customer Service.

**Setup of the session**

The product **BCR-ABL P190 ELITe MGB® Kit** in association to the **ELITe InGenius** can be used in in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Calibration run (PCR only),
D. Amplification run for Positive and Negative Control (PCR only).

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

**Note:** The **ELITe InGenius** system can be linked to the "Location Information Server" (LIS) through which it is possible to send the work session information. Refer to the instrument user's manual for more details.

Before starting the session, it is mandatory to do the following:

1. Thaw for 30 minutes at room temperature (+18 / 25 °C) the **P190 PreMix** (WHITE cap) and the **ABL PreMix** (NEUTRAL cap) test tubes needed for the session, remembering that the content of each test tube is enough for **36 reactions**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice.

2. Thaw for 30 minutes at room temperature (+18 / 25 °C) the **PCR MasterMix** (NEUTRAL cap) test tubes needed for the session, remembering that the content of each test tube is enough for **36 reactions**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice.

3. Take the **RT EnzymeMix** (cap with BLACK insert) tubes necessary for the session remembering that the content of each tube is sufficient to set up **36 reactions**. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep in ice.

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

4. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker.

5. Calculate the volumes of the three components provided by kit that are needed for preparing the **complete reaction mixture:**

a. For the Calibration follow the table below:

| Target | Number of samples | PreMix | PCR MasterMix | RT EnzymeMix |
|---|---|---|---|---|
| P190 | 5 | 30 µL | 90 µL | 0.9 µL |
| ABL | 3 | 20 µL | 60 µL | 0.6 µL |

b. for Controls and samples follow the table below:

| Number of samples | P190 or ABL PreMix | PCR MasterMix | RT EnzymeMix |
|---|---|---|---|
| 1 | 15 µL | 45 µL | 0.5 µL |
| 2 | 25 µL | 75 µL | 0.8 µL |
| 3 | 40 µL | 120 µL | 1.2 µL |

6. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the three components.

7. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

**Note:** The **complete reaction mixture** should be used within **5** hours when kept on board in the refrigerated block. The complete reaction mixture **cannot** be stored. This time allows to carry out 1 work session of 3.5 hours and to start a second work session.

The main operations for setting the four types of travel are described below.

**A. Integrated run**

To set up the integrated run starting from pre-treated samples, carry out the steps below following the GUI:

1. Select “Perform Run” from the “Home” screen.
2. Ensure that the “Extraction Input Volume” is 200 µL and that the “Extracted Elute Volume” is 100 µL.
3. For each Track of interest fill in the “SampleID” (SID) by typing or by scanning the sample barcode.
4. Select the assay protocol to be used in the “Assay” column (i.e. BCR-ABL P190 ELITe_PBL_200_100).
5. Ensure that the “Protocol” displayed is: “Extract + PCR”.
6. Ensure the sample loading position in the “Sample Position” column is “Extraction Tube (bottom row)”. Click “Next” to continue the setup.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassette”, the “ELITe InGenius SP RNA” extraction cartridges and the “ELITe InGenius DNase I“, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click “Next” to continue the setup.



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10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

**Note:** At the end of the run the remaining Extracted Sample in the “Elution tube” must be removed from the instrument, capped, identified and stored at -20 °C for one month. Avoid spilling Extracted Sample.

**Note:** At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

**Note:** At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

**B. Amplification run**

To set up the amplification run starting from extracted RNA, carry out the following steps as per GUI:

1. Select “Perform Run” from the “Home” screen.
2. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
3. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
4. Select the assay protocol to be used in the “Assay” column (i.e. BCR-ABL P190 ELITe_PBL_200_100).
5. Select “PCR Only” in the “Protocol” column.
6. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassettes” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

**Note:** At the end of the run the remaining Extracted Sample in the “Elution tube” must be removed from the instrument, capped, identified and stored at -20 °C for one month. Avoid spilling Extracted Sample.

**Note:** At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

**Note:** At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

**C. Calibration run**

To set up the Calibration run for Q-PCR Standards, carry on the steps below following the GUI:

1. Thaw a tube of each BCR-ABL P190 Q - PCR Standard levels for P190 calibration (Cal1: BCR-ABL Q-PCR Standards $10^1$, Cal2: BCR-ABL Q-PCR Standards $10^2$, Cal3: BCR-ABL Q-PCR Standards $10^3$, Cal4: BCR-ABL Q-PCR Standards $10^4$, Cal5: BCR-ABL Q-PCR Standards $10^5$). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.

2. Thaw another tube of BCR-ABL P190 Q - PCR Standard $10^5$, $10^4$ and $10^3$ for ABL calibration (Cal3: BCR-ABL Q-PCR Standards $10^3$, Cal4: BCR-ABL Q-PCR Standards $10^4$, Cal5: BCR-ABL Q-PCR Standards $10^5$). Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
5. For P190 calibration, select the Assay Protocol “BCR-ABL P190 ELITe_STD_P190” in the “Assay” column and fill in the lot number and expiry date of **BCR-ABL P190 Q-PCR Standard**.
6. For ABL calibration, select the Assay Protocol “BCR-ABL P190 ELITe_STD_ABL” in the “Assay” column and fill in the lot number and expiry date of **BCR-ABL P190 Q-PCR Standard**.
7. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load the “PCR Cassettes” and the **BCR-ABL P190 Q-PCR Standard** tubes following the GUI instruction. Click “Next” to continue the setup.
10. Close the instrument door.
11. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

**Note:** At the end of the run the remaining **BCR-ABL P190 Q-PCR Standard** must be removed from the instrument, capped and stored at -20 °C.

**Note:** At the end of the run the PCR Cassettes with the reaction products and the consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid spilling the reaction products.

**Note:** At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

**D. Amplification run for Positive Control and Negative Control**

To setup the amplification run for Positive Control and Negative Control, carry out the following steps as per GUI:

1. Thaw BCR-ABL P190 - ELITe Positive Control tube for the session. Each tube is sufficient for 2 sessions. Mix gently, spin down the content for 5 seconds.
2. Transfer at least 80 µL of molecular biology grade water to an “Elution tube”, provided with the ELITe InGenius SP 200 Consumable Set.
3. Select “Perform Run” from the “Home” screen.
4. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
5. In the Track of interest, select the Assay protocol to be used in the “Assay” column.
6. For the positive control, select the Assay Protocol “BCR-ABL P190 ELITe_PC” in the “Assay” column and fill in the lot number and expiry date of BCR-ABL P190 Positive Control.
7. For the negative control, select the Assay Protocol “BCR-ABL P190 ELITe_NC” in the “Assay” column and fill in the lot number and expiry date of the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.



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10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR cassettes”, the BCR-ABL P190 Positive Control tube and the Negative Control tube following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows the user to view, approve, store the results and to print and save the report.

**Note:** At the end of the run the remaining **BCR-ABL P190 Positive Control** must be removed from the instrument, capped and stored at -20 °C. The remaining Negative Control must be disposed.

**Note:** At the end of the run the PCR Cassettes with the reaction products and consumables must be removed from the instrument and disposed of without environmental contaminations. Avoid spilling the reaction products.

**Note:** At the end of the run, the **complete reaction mixture** can be kept on board in the refrigerated block taking into account the maximum time of 5 hours.

**Review and approval of results**

At the end of the run, the “Results Display” screen is automatically shown. In this screen the sample / Calibrator / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports (“Sample Report” or “Track Report”). Refer to the instrument user’s manual for more details.

**Note:** The **ELITe InGenius** can be linked to the “Laboratory Information Server” (LIS) through which it is possible to send the work session results to the laboratory data centre. Refer to the instrument user's manual for more details.

The **ELITe InGenius** generates results using the **BCR-ABL P190 ELITe MGB® Kit** through the following procedure:

A. Validation of Calibration curve,
B. Validation of amplification Positive Control and Negative Control results,
C. Validation of sample results,
D. Sample result reporting.

**A. Validation of Calibration curve**

The fluorescence signals emitted by the probe for P190 (Channel 1 "P190") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “BCR-ABL ELITe_STD_P190”.

The fluorescence signals emitted by the probe for ABL (Channel 1 "ABL") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “BCR-ABL ELITe_STD_ABL”.

The P190 and ABL Calibration curves, specific for the amplification reagent lot, is stored in the database (Calibration). It can be viewed and approved by personnel qualified as “Administrator” or “Analyst” following the GUI instructions.

The Calibration curves, specific for the amplification reagent lot, will expire **after 60 days**.

**Note:** If the Calibration curve does not meet the acceptance criteria, the “Failed” message is shown on the “Calibration” screen and it is not possible to approve the curve. The Calibrator amplification reactions must be repeated.

**Note:** If the Calibration Curve is run together with samples and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

### B. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probe for P190 (Channel 1 "P190") in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol "BCR-ABL P190 ELITe_PC" and "BCR-ABL P190 ELITe_NC".

The amplification Positive Control and Negative Control results, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

The results of Positive Control and Negative Control amplification are used by the instrument software to calculate and setup the "Control Charts". Four Positive Control and Negative Control results, from 4 different runs are requested to set up the "Control Chart". After that, the results of Positive control and Negative Control are used for monitoring the amplification step performances. Refer to the user's manual of the instrument for more details.

**Note:** If the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the "Failed" message is shown on the "Controls" screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction must be repeated.

**Note:** If the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

### C. Validation of Samples results

The fluorescence signals emitted by the probe for P190 (Channel 1 "P190") and by the probe for ABL (Channel 1 "ABL") in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol BCR-ABL P190_PBL_200_100.

Results are shown in the reports generated by the instrument ("Result Display").

The sample run can be approved when the three conditions reported in the table below are met.

| **1) Calibration curve** | **Status** |
|---|---|
| BCR-ABL P190 Q-PCR Standard | APPROVED |
| **2) Positive Control** | **Status** |
| BCR-ABL P190 Positive Control | APPROVED |
| **3) Negative Control** | **Status** |
| BCR-ABL P190 Negative Control | APPROVED |

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe InGenius software** algorithm and the Assay protocol parameters and explains in the following paragraph.

In case of the amplification reactions of each **sample**, **P190 Ct** values are used to detect and quantify the presence of target mRNA, while **ABL Ct** values are used to detect and quantify the presence of control mRNA (extraction validation and target normalization).

The **P190 Ct** and **ABL Ct** values in the amplification reactions of each **sample** and the **Standard Curves** are used to calculate the **Quantity of mRNA** of P190 and ABL present in the amplification reactions of the samples. Then the **Quantities of mRNA** of P190 and ABL are used to calculate the **percentage of P190** mRNA copies normalized to ABL mRNA copies (**%P190**).



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The possible result messages of a Sample are listed the table below.

| Result of Sample run | Interpretation |
|---|---|
| P190:percentage is x.xxxx% | **P190 RNA was detected.** Calculated %P190 value is shown. |
| P190:percentage is 0.0000% | **P190 RNA was not detected or it is below the Limit of Detection** of the assay. Equivalent to %P190 = 0%. |
| Inconclusive - Retest Sample | **P190 RNA was detected but %P190 cannot be calculated.** Differences in P190 quantities within the duplicate are not acceptable. Retest the sample. |
| Invalid - Retest Sample | **ABL RNA was below the Cut-off (10,000 copies).** Retest the sample. |

To complete the information for each sample analyzed, the results of single reactions (Tracks) for P190 and ABL targets are reported as follow.

| Result of single Replicate | Interpretation |
|---|---|
| P190: RNA Detected, quantity equal to xxx copies/reaction | **P190 RNA was detected.** Calculated Quantity of mRNA of P190 is shown. |
| P190: RNA Not detected or below the LoD | **P190 RNA was not detected or it is below the Limit of Detection** of the assay. |
| ABL: RNA Detected, quantity equal to xxx copies/reaction | **ABL RNA was detected**. Calculated Quantity of mRNA of ABL is shown. |
| ABL: RNA Not detected or below the LoD | **ABL RNA was not detected or it is below the Limit of Detection** of the assay. |

The following table that shows the different cases that might occur in an amplification session and the approach to generate the result messages.

| Sample | P190 (copies/reaction) | ABL (copies/reaction) | Result of Sample run (%P190) | Interpretation |
|---|---|---|---|---|
| **1st replicate** | **Quantity** | **Quantity ≥ 10,000** | **P190 percentage is x.xxxx%** | **P190 RNA was detected.** Calculated %P190 value is shown. |
| **2nd replicate** | **Quantity** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **Not Detected** | **Quantity ≥ 10,000** | **P190 RNA Not Detected or below the LoD** | **P190 RNA was not detected or it is below the Limit of Detection** of the assay. Equivalent to %P190 = 0% |
| **2nd replicate** | **Not Detected** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **Quantity < 10 copies** | **Quantity ≥ 10,000** | **P190 percentage is x.xxxx %** | **P190 RNA was detected.** Calculated %P190 value is shown. |
| **2nd replicate** | **Not Detected** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **Quantity > 10 copies** | **Quantity ≥ 10,000** | **Inconclusive-Retest Sample** | **P190 RNA was detected but %P190 cannot be calculated.** Differences in P190 quantities within the duplicate are not acceptable. Retest the sample. |
| **2nd replicate** | **Not Detected** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **Detected Or Not Detected** | **Quantity < 10,000** | **Invalid-Retest Sample** | **ABL RNA was below the Cut-off (10,000 copies).** Retest the sample. |
| **2nd replicate** | **Detected Or Not Detected** | **Quantity ≥ 10,000** | | |

| Sample | P190 (copies/reaction) | ABL (copies/reaction) | Result of Sample run (%P190) | Interpretation |
|---|---|---|---|---|
| 1st replicate | Detected Or Not Detected | Quantity < 10,000 | Invalid-Retest Sample | ABL RNA was below the Cut-off (10,000 copies). Retest the sample. |
| 2nd replicate | Detected Or Not Detected | Quantity < 10,000 | | |

**Note:** if for a sample the result of the P190 amplification reaction is < 3 copies/reaction, the quantity will be reported to 3 copies/reaction.

Samples reported as "Invalid - Retest Sample" by the ELITe InGenius software are not suitable for result interpretation as the ABL mRNA was not detected efficiently. In this case, problems have occurred during the extraction phase (loss of RNA, presence of inhibitors or degradation of extracted RNA, see Troubleshooting) which may cause incorrect and false negative results. The sample is not suitable for calculation of %P190, the assay is invalid and must be repeated on extracted RNA first and, if a problem is confirmed, start from the extraction of a new sample.

Samples reported as "Inconclusive-Retest Sample" by the ELITe InGenius software are not suitable for result interpretation as the P190 mRNA was not detected efficiently. In this case, problems have occurred during the extraction phase (loss of RNA, presence of inhibitors or degradation of extracted RNA, see Troubleshooting) which may cause incorrect and false negative results. The sample is not suitable for calculation of %P190, the assay is inconclusive and must be repeated on extracted RNA first and, if a problem is confirmed, start from the extraction of a new sample.

Samples reported as "P190 RNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect P190 RNA. In this case it cannot be excluded that the RNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics").

**Note:** The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

The Sample run results are stored in the database and, if valid, can be approved (Result Display) by personnel qualified as "Administrator" or "Analyst", following the GUI instruction. From the Result Display" window it is possible to print and save the Sample run results as "Sample Report" and "Track Report".

**D. Samples result reporting**

The sample results are stored in the database and can be viewed as "Sample Report" and "Track Report".

The "Sample Report" shows the details of a work session stored by selected sample (SID).

The "Track Report" shows the details of a work session by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.



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## PERFORMANCE CHARACTERISTICS

**Limit of Detection**

The P190 Limit of Detection of the assay with total RNA was verified using the reference calibrated material IVS-0032 Clonal Control RNA (InVivoScribe, US), total RNA extracted from a human cell line positive for BCR-ABL P190 e1a2 diluted in total RNA from a human cell line negative for the translocation. The dilution $10^{-4.5}$ was tested in 40 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the ELITe InGenius system.

The final results are summed up in the following table.

| Limit of Detection with total RNA samples and ELITe InGenius | | | | | |
|---|---|---|---|---|---|
| **Sample** | **Dilution** | **N** | **Positive** | **Negative** | **P190%** |
| P190 RNA | $10^{-4.5}$ | 40 | 39 | 1 | 0.0032% |

All replicates resulted positive for P190, with a mean concentration of P190% equal to 0.0032%. The mean quantity of ABL recorded in the tests for the definition of the Limit of Detection was approximately 120,000 copies per reaction.

**Linear measuring range**

The P190 linear measuring range of this assay with total RNA was determined using the panel of reference calibrated material IVS-0032 Clonal Control RNA (InVivoScribe, US). The panel consisted of total RNA extracted from a human cell line positive for BCR-ABL P190 e1a2diluted in total RNA from a human cell line negative for the translocation. The dilutions ranged from pure P190 positive RNA (P190 RNA) to $10^{-5}$ (1 Log dilution steps). Each sample of the panel was tested in 4 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the ELITe InGenius system. The statistical analysis was performed by linear regression.

The analysis of the data obtained demonstrated that the assay has a linear response for the panel points from pure P190 positive RNA to $10^{-5}$ with a linear correlation coefficient greater than 0.99.

The upper limit of the linear measurement verified in this test is the pure P190 positive RNA, corresponding to a concentration of P190% equal to 100%.

The lower limit of the linear measurement verified in this test is the dilution of $10^{-5}$, lower than the Limit of Detection and corresponding to a concentration of P190% equal to 0.001%.

The final results are summed up in the following table.

| Linear measuring range with total RNA samples and ELITe InGenius | | | |
|---|---|---|---|
| Sample | Mean P190 copies / reaction | Mean P190 Log copies /reaction | Std Dev |
| P190 RNA | 346,796 | 5.540 | 0.01 |
| $10^{-1.0}$ dilution | 34,073 | 4.532 | 0.02 |
| $10^{-2.0}$ dilution | 3,453 | 3.530 | 0.10 |
| $10^{-3.0}$ dilution | 474 | 2.675 | 0.034 |
| $10^{-4.0}$ dilution | 74 | 1.860 | 0.094 |
| $10^{-5.0}$ dilution | 4 | 0.590 | n.a. |

The mean quantity of ABL recorded in the tests was approximately 180,000 copies per reaction.

**Diagnostic sensitivity: confirmation of positive samples**

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested analysing a panel of P190 positive clinical samples.

The diagnostic sensitivity was evaluated using 24 fresh specimens of peripheral blood collected in EDTA from leukemia patients tested positive for BCR-ABL translocation, variant P190 with a CE-IVD real time amplification product. Each sample was tested with **ELITe InGenius** in "Extract + PCR" mode.

The final results are summed up in the following table:

| Samples | N | positive | negative |
|---|---|---|---|
| Peripheral blood samples positive for P190 | 24 | 24 | 0 |

In the test, 24 out of 24 samples were confirmed. In this test the diagnostic sensitivity of the assay was equal to 100%.

The mean quantity of ABL recorded in the tests was approximately 70,000 copies per reaction.

**Diagnostic specificity: confirmation of negative samples**

The diagnostic specificity of the assay, as confirmation of negative samples, was tested analysing a panel of P190 negative clinical samples.

The diagnostic specificity was evaluated using 30 fresh specimens of peripheral blood collected in EDTA from different subjects tested negative for BCR-ABL translocation, variant P190 with a CE-IVD real time amplification product. Each sample was tested with **ELITe InGenius** in "Extract + PCR" mode.

The final results are summed up in the following table:

| Samples | N | positive | negative |
|---|---|---|---|
| Peripheral blood samples negative for P190 | 30 | 1 | 29 |

In the test, 29 out of 30 samples were confirmed, one sample gave a discrepant positive result. In this test the diagnostic specificity of the assay was equal to 96.7%.

The mean quantity of ABL recorded in the tests was approximately 50,000 copies per reaction.

**N.B.:** The complete data and results of the tests carried out to evaluate the performance characteristics of the product with matrices and instruments are recorded in the Product Technical File " BCR-ABL P190 ELITe MGB® Kit", FTP G07PLD190.



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## ABI 7500 Fast Dx Real-Time PCR Instrument
## ABI 7300 Real-Time System

## SAMPLES AND CONTROLS

**Samples**

This product must be used with **RNA extracted** from the following clinical samples: lymphomonocyte and leukocyte suspensions from peripheral blood collected in EDTA or sodium citrate, bone marrow blood collected in EDTA or sodium citrate.

This product must be used adding up from 300 ng to 1.5 µg of **extracted RNA** to the reverse transcription and real time amplification reaction.

Suspensions of lymphomonocytes and leukocytes.

The suspensions of lymphomonocytes or leukocytes (e.g. buffy coat), used for RNA extraction, must be prepared from clinical samples of peripheral blood or bone marrow according to laboratory guidelines, resuspended in sterile physiological solution or sterile PBS and stored at +2 / 8 °C for a maximum of four hours.

The optimal quantity of lymphomonocytes or leukocytes from which to extract total RNA is approximately 10,000,000 cells.

Do not freeze suspensions of lymphomonocytes or leukocytes in order to avoid degradation of RNA.

The peripheral blood collected in EDTA or sodium citrate or bone marrow blood collected in EDTA or sodium citrate, used for lymphomonocytes or leukocytes preparation, must be collected according to laboratory guidelines, transported at +2 / 8 °C and stored at +2 / 8 °C for a maximum of four hours.

Do not freeze peripheral blood or bone marrow in order to prevent degradation of RNA.

**Interfering substances**

The extracted RNA must not contain heparin, haemoglobin, Ficoll®, ethanol or 2-propanol in order to prevent inhibition and frequent invalid results.

Quantities of RNA more than 1.5 µg per reaction could inhibit the reverse transcription reaction and the real time amplification.

Quantities of human genomic DNA higher than 100 ng per reaction in the RNA extracted from the sample could inhibit the reverse transcription reaction and the real time amplification.

There is no data available concerning inhibition caused by antibiotics, antiviral drugs, chemotherapeutic or immunosuppressant drugs.

**Amplification controls**

It is absolutely mandatory to validate each amplification session with a negative reaction control and a positive reaction control.

As a negative control (NC), use molecular biology grade water (not supplied with the product). This is to be added to the reaction in place of the RNA extracted from the sample.

As positive control (PC), the **«BCR ABL P190 ELITe Standard»** product is used.

**Quality controls**

It is recommended to validate the whole analysis procedure of each extraction, reverse transcription and amplification session by processing a negative sample and a positive sample that has previously been tested or reference calibrated material.

## PROCEDURE

**Setting of the real time amplification session**
(To perform in the amplification / detection area)

Using **7300 Real-Time PCR System** or **7900 Real-Time PCR System** instrument.

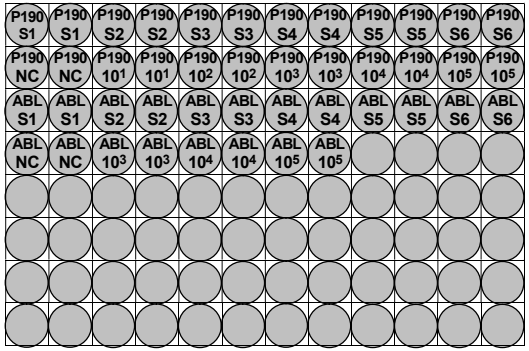
Before starting the session, referring to the instrument documentation, follow the instruction below:
- switch on the real time thermal cycler, switch on the control computer, launch the software and open an "absolute quantification" session,
- set (Detector Manager) the "detector" for the P190 probe with the “reporter” = "FAM" and the "quencher" = “none " (non fluorescent) " and name it "P190",
- set (Detector Manager) the "detector" for the ABL probe with the “reporter” = "FAM" and the "quencher" = “none” (non fluorescent) and name it "ABL",
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence to be measured), the "passive reference" = "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the complete reaction mixture and samples into the wells.

**Note:** in order to determine the RNA titre in the starting sample, set up in duplicate the reactions with the **Q - PCR Standard** and the two complete reaction mixture to obtain the two standard curves, one for P190 ($10^5$, $10^4$, $10^3$, $10^2$, $10^1$ copies / reaction) and one for ABL ($10^5$, $10^4$, $10^3$ copies / reaction).

**Note:** To optimize the use of the product, the standard curve for P190 can be set up omitting the Q - PCR Standard level $10^1$ copies / reaction and using the other 4 Q - PCR Standard levels ($10^5$, $10^4$, $10^3$, $10^2$ copies / reaction) or using the Q - PCR Standard level $10^1$ copies / reaction and omitting the Q - PCR Standard level $10^3$ copies / reaction ($10^5$, $10^4$, $10^2$, $10^1$ copies / reaction).

**Note:** calculate, for the target P190 and the control ABL, two wells for each sample to be analyzed (S), two wells for the negative control amplification (NC) and two wells for each Q - PCR Standard (5 o 4 points for P190 and 3 points for ABL).

Below is an example of how the analysis of 6 samples can be organized.




**Key:**

**P190 S1** - **P190 S6**: P190 reactions with the test samples,

**P190 NC**: P190 reaction with the negative control,

**P190 $10^1$**, **P190 $10^2$**, **P190 $10^3$**, **P190 $10^4$**, **P190 $10^5$**, P190 reactions with the DNA standard $10^1$, $10^2$,$10^3$, $10^4$ and $10^5$ copies / reaction.

**ABL S1** - **ABL S6**: ABL reactions with the test samples,

**ABL NC**: ABL reactions with the negative amplification control,

**ABL $10^3$**, **ABL $10^4$**, **ABL $10^5$**, ABL reactions with the DNA standard $10^3$, $10^4$ and $10^5$ copies / reaction.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal



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Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to the amplification stage a step (Add Step) for **extension at 72 °C**;

**Note:** the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the **hybridization at 56 °C step**.

- modify timing as indicated in the table **"Thermal cycle"**,
- set the cycle number to **45**,
- set the reaction volume to **30 µL**,

| Thermal cycle | | |
|---|---|---|
| **Phase** | **Temperature** | **Time** |
| Reverse-transcription | 50 °C | 20 min. |
| Initial denaturation | 94 °C | 5 min. |
| Amplification and detection (45 cycles) | 94 °C | 10 sec. |
| | 56 °C (fluorescence acquisition) | 30 sec. |
| | 72 °C | 15 sec. |

When **7500 Fast Dx Real-Time PCR Instrument** is used.

Before starting the session, referring to the instrument documentation, follow the instruction below:
- switch on the real time thermal cycler, switch on the control computer, launch the software, open an "absolute quantification" session and set "Run mode: Fast 7500",
- set (Detector Manager) the "detector" for the P190 probe with the “reporter” = "FAM" and the "quencher" = “none " (non fluorescent) " and name it "P190",
- set (Detector Manager) the "detector" for the ABL probe with the “reporter” = "FAM" and the "quencher" = “none” (non fluorescent) and name it "ABL",
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "CY5" (AP593 is used instead of CY5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the complete reaction mixture and samples into the wells.

**Note:** in order to determine the RNA titre in the starting sample, set up two series of reactions with the **Q - PCR standard** to obtain the two **standard curves**, one for P190 ($10^5$, $10^4$, $10^3$, $10^2$,$10^1$ copies / reactions) and one for ABL ($10^5$, $10^4$, $10^3$ copies / reaction).

**Note:** To optimize the use of the product, the standard curve for P190 can be set up omitting the Q - PCR Standard level $10^1$ copies / reaction and using the other 4 Q - PCR Standard levels ($10^5$, $10^4$, $10^3$, $10^2$ copies / reaction) or using the Q - PCR Standard level $10^1$ copies / reaction and omitting the Q - PCR Standard level $10^3$ copies / reaction ($10^5$, $10^4$, $10^2$, $10^1$ copies / reaction)

**Note:** calculate, for the target P190 and the control ABL, two wells for each sample to be analyzed (S), two wells for the negative control amplification (NC) and two wells for each Q - PCR Standard (5 or 4 points for P190 and 3 points for ABL).

The set up of the quantitative analysis of 6 samples is shown, by way of example, in the previous paragraph describing the procedure for the **7300 Real Time PCR System** instrument.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to the amplification stage a step (Add Step) for **extension step at 72 °C**,

**Note:** the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the **hybridization step at 56 °C**.

- modify time as indicated in the table **"Thermal cycle"**,
- set the cycle number to **45**,
- set the reaction volume to **30 µL**.

| Thermal cycle | | |
|---|---|---|
| **Phase** | **Temperature** | **Time** |
| Reverse-transcription | 50 °C | 20 min. |
| Initial denaturation | 94 °C | 5 min. |
| Amplification and detection (45 cycles) | 94 °C | 10 sec. |
| | 56 °C (fluorescence acquisition) | 30 sec. |
| | 72 °C | 15 sec. |

**Amplification set-up**
(To be performed in the extraction / preparation area)

Before starting the session, follow the instruction below:
- verify the availability of requested reagents for each sample to be analyzed (see table on page 10).
- take and thaw at room temperature (+18 / 25 °C) the test tubes containing the RNA samples to be analysed. Vortex the tubes for 5 seconds, spin down the content for 5 seconds and keep them in a cold block,
- take and thaw the **P190 PreMix** (PURPLE cap) and **ABL PreMix** (NEUTRAL cap) test tubes needed for the session for 30 minutes at room temperature (+18 / 25 °C). Remember that the content of each test tube is enough for **50 reactions**. Vortex the tubes for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block,
- take and thaw (+18 / 25 °C) the **PCR MasterMix** (NEUTRAL cap) tubes necessary for the session for 30 minutes at room temperature (+18 / 25 °C). Remember that the content of each tube is sufficient to set up **50** reactions. Vortex the tubes for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block,
- take the **RT EnzymeMix** (BLACK cap) tubes necessary for the session remembering that the content of each tube is sufficient to set up **50 reactions**. Centrifuge for 5 seconds to bring the contents to the bottom and keep in a cold block,

**Note:** The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

- take and thaw the **P190-ABL Q-PCR Standard** tubes necessary for the session (**for both reactions P190 and ABL**) for 30 minutes at room temperature (+18 / 25 °C). Remember that the contents of each test tube are sufficient to set up **12 reactions**. Vortex the tubes for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block,
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powderless gloves and not to damage the wells,
- take the **Amplification Sealing Sheet** that will be used during the session, being careful to handle it with powderless gloves and not to damage it,
- prepare two 1.5 mL sterile polypropylene tubes (not provided with this product): one for the complete reaction mixture of **P190** and the other for the complete reaction mixture **ABL** and mark them in a recognizable manner with a permanent marker,
- prepare two complete reaction mixtures, one for **P190** and the other for **ABL**, using the three components provided in the product, based on the number of samples to be analyzed, as described in the following table.



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**Note:** For preparing one reverse transcription and real time amplification reaction 5 µL of PreMix, 15 µL of PCR MasterMix and 0,3 µL of RT EnzimeMix are needed. The volumes indicated in the table are sufficient for the set up of the reactions for reverse transcription and real time amplification required for the number of samples to be tested, negative control and four Q-PCR Standards, in duplicate plus an adequate safety margin.

| Number of samples | PreMix | PCR MasterMix | RT EnzymeMix |
|---|---|---|---|
| 1 | 65 µL | 195 µL | 3.9 µL |
| 2 | 75 µL | 225 µL | 4.5 µL |
| 3 | 85 µL | 255 µL | 5.1 µL |
| 4 | 95 µL | 285 µL | 5.7 µL |
| 5 | 110 µL | 330 µL | 6.6 µL |
| 6 | 120 µL | 360 µL | 7.2 µL |
| 7 | 130 µL | 390 µL | 7.8 µL |
| 8 | 140 µL | 420 µL | 8.4 µL |
| 9 | 150 µL | 450 µL | 9.0 µL |
| 10 | 160 µL | 480 µL | 9.6 µL |
| 11 | 170 µL | 510 µL | 10.2 µL |
| 12 | 180 µL | 540 µL | 10.8 µL |
| 13 | 190 µL | 570 µL | 11.4 µL |
| 14 | 205 µL | 615 µL | 12.3 µL |
| 15 | 215 µL | 645 µL | 12.9 µL |
| 16 | 225 µL | 675 µL | 13.5 µL |
| 17 | 235 µL | 705 µL | 14.1 µL |
| 18 | 245 µL | 735 µL | 14.7 µL |
| 19 | 255 µL | 765 µL | 15.3 µL |

Vortex the two complete reaction mixtures for 10 seconds three times, spin down the content for 5 seconds and keep them in a cold block.

**Note:** The complete reaction mixtures prepared should be used within 1 hour. The reaction mixtures prepared **cannot** be stored.

Set up **P190 and ABL reactions** as described below taking care to keep the **Amplification microplate** in a cold block (~ +5 °C).

1. Accurately pipet **20 µL** of **"P190" complete reaction mixture** into the bottom of the **"P190" Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.
2. Accurately pipet **20 µL** of **"ABL" complete reaction mixture** into the bottom of the **"ABL" Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.
3. Accurately pipet **10 µL** of **RNA extract** into the complete reaction mixture from the first sample in the two corresponding wells of "**P190**" and in the two corresponding wells of "**ABL**" of the **Amplification microplate**, as previously established in the **Work Sheet**. Mix the sample well by pipetting the **extracted RNA** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface. Proceed in the same way with all the other samples of **extracted RNA**.
4. Accurately pipet **10 µL** of **Molecular biology grade water** (not provided with this product) into the complete reaction mixture in the two corresponding wells of "**P190**" and in the two corresponding wells of "**ABL**" of **Amplification microplate**, as previously established in the **Work Sheet**. Mix the negative control well by pipetting the **Molecular biology grade water** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface.

5. Accurately pipet **10 µL** of the first **P190-ABL Q-PCR Standard** into the complete reaction mixture in the two corresponding wells of "**P190**" of **Amplification microplate**, as previously established in the **Work Sheet**. Mix the standard well by pipetting the **P190-ABL Q-PCR Standard** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface. Proceed in the same way with the other **P190-ABL Q-PCR Standards**.
6. Accurately pipet, **10 µL** of the first **P190-ABL Q-PCR Standard** into the complete reaction mixture in the two corresponding wells of "**ABL**" of **Amplification microplate**, as previously established in the **Work Sheet**. Mix the standard well by pipetting the **P190-ABL Q-PCR Standard** into the complete reaction mixture three times. Avoid creating bubbles both at the well bottom and in surface. Proceed in the same way with the other **P190-ABL Q-PCR Standards.**
7. Accurately seal the **Amplification microplate** with the **Amplification Sealing Sheet**.
8. Transfer the **Amplification microplate** into the real time thermal cycler in the amplification / detection area and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name (e.g. "year-month-day-BCR-ABL-P190-EGSpA").

**Note:** At the end of the thermal cycle the **Amplification microplate** containing the reaction products must be removed from the instrument and disposed of without producing environmental contamination. In order to avoid spilling the reaction products, the **Amplification Sealing Sheet must not be removed from the Amplification microplate**.



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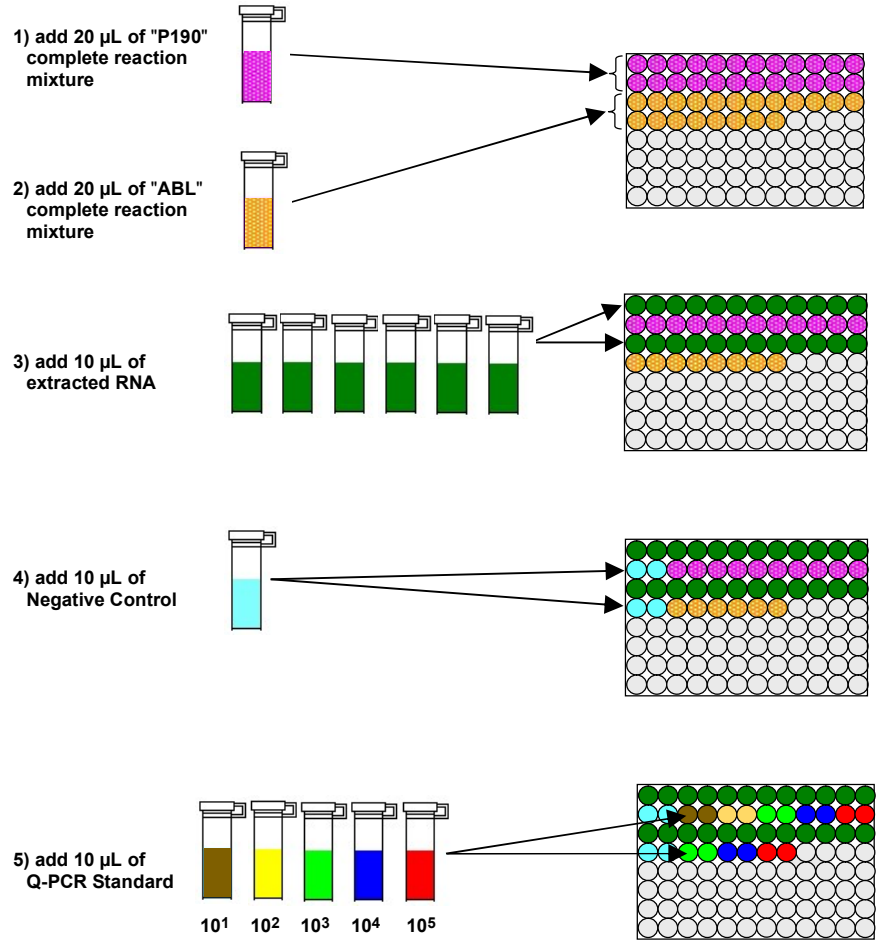
The following picture summarizes the setup of the reverse-transcription and real-time amplification reactions for P190 e ABL.



**Analysis of the results**

The values of fluorescence emitted by the specific probe for P190 (FAM detector "P190") in the P190 amplification reaction and by the specific probe for ABL (FAM detector "ABL") in the ABL amplification reaction must be analysed by the instrument software.

Before analysing, referring to the instrument documentation, it is necessary to:
- manually set (Results > Amplification plot > delta Rn vs Cycle) the calculation range for the **fluorescence background level (Baseline)** from cycle 6 to cycle 15,

**Note:** in the case of a positive sample with a high titre of P190 or ABL, the FAM fluorescence of the specific probe for P190 or ABL may begin to increase before the 15$^{th}$ cycle. In this case the calculation range for the "baseline" must be set for both detectors from cycle 6 to the cycle in which the FAM fluorescence starts to increase as detected from the instrument software (Results > Component).

- manually set the **Threshold** for the FAM detector "P190" to **0.1**,
- manually set the **Threshold** for the FAM detector "ABL" to **0.1**.

The values of fluorescence emitted by the specific probes in the amplification reactions and the **Threshold** value of fluorescence are used to determine the **Threshold Cycle (Ct)**, the cycle at which the fluorescence signal reaches the threshold value.

**Standard Curve**

In case of the P190 and ABL amplification reaction of the **Q - PCR Standards**, the **P190 and ABL Ct** values are used to calculate the two **Standard Curves** (Results > Standard Curve) of the amplification session and to validate the amplification and detection as shown in the following table:

| P190 Reaction - Q - PCR Standard $10^5$ detector FAM "P190" | Assay result | Amplification / Detection |
|---|---|---|
| Ct ≤ 25 | POSITIVE | CORRECT |

| P190 Reaction - Standard Curve detector FAM "P190" | Acceptance range* | Amplification / Detection |
|---|---|---|
| Determination coefficient (R2) | 0.980 ≤ R2 ≤ 1.000 | CORRECT |

| ABL Reaction - PCR Standard $10^5$ detector FAM "ABL" | Assay result | Amplification / Detection |
|---|---|---|
| Ct ≤ 25 | POSITIVE | CORRECT |

| ABL Reaction - Standard Curve detector FAM "ABL" | Acceptance range | Amplification / Detection |
|---|---|---|
| Determination coefficient (R2) | 0.990 ≤ R2 ≤ 1.000 | CORRECT |

***Note:** If the standard curve for P190 has been set up omitting Q - PCR Standard level $10^1$ copies / reaction the acceptance range of the Determination Coefficient will be 0.990 ≤ R2 ≤ 1.000.

If the result of the **Q - PCR Standard $10^5$** amplification reaction is **Ct > 25** or **Ct Undetermined** or if the **Determination coefficient** (**R2**) value does not fall within the limits, the target DNA has not been correctly detected. This means that problems occurred during the amplification or the detection step (incorrect preparation of the complete reaction mix, incorrect dispensing of the complete reaction mix or of the standards, degradation of the probe or of the standards, incorrect setting of the standard position, incorrect setting of the thermal cycle, see Troubleshooting), which may lead to incorrect results. The session is not valid and has to be repeated starting from the amplification step.



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**Negative Control**

In case of the P190 and ABL amplification reaction of the **Negative Control**, the **P190 and ABL Ct** values (Results > Report) are used to validate amplification and detection as shown in the following table:

| P190 Reaction - Negative Control detector FAM "P190" | Assay result | Amplification / Detection |
|---|---|---|
| Ct Undetermined | NEGATIVE | CORRECT |

| ABL Reaction - Negative Control detector FAM "ABL" | Assay result | Amplification / Detection |
|---|---|---|
| Ct Undetermined | NEGATIVE | CORRECT |

If the result of the **Negative control** amplification reaction is different than **Ct undetermined** for P190 and ABL, it means that target DNA has been detected in the amplification reaction. Problems have occurred during the amplification phase (contamination, incorrect preparation of the complete reaction mix, degradation of the probe, incorrect setting of the negative control position, incorrect setting of the thermal cycle, see Troubleshooting) which may cause incorrect results and false positives. The session is invalid and must be repeated from the amplification phase.

**Samples**

In case of amplification reactions of each **sample**, **P190 Ct** values are used to detect and quantify the presence of target mRNA, while **ABL Ct** values are used to detect and quantify the presence of control mRNA (extraction validation and target normalization).

**Note:** Verify by using the software tools of the instruments (Results > Amplification plot > delta Rn vs Cycle) that the **Ct** is determined by a rapid and regular increase of fluorescence values and not by isolated peaks or background signal increases.

The **P190 Ct** and **ABL Ct** values in the amplification reactions of each **sample** and the **Standard Curves** of the amplification session are used to calculate the **Quantity of mRNA** of P190 and ABL present in the amplification reactions of the samples.

| Sample reactions | | |
|---|---|---|
| **Detector FAM** | **mRNA** | **Quantity of mRNA obtained** |
| Ct determined | DETECTED | Quantity |
| Ct Undetermined | NOT DETECTED | 0 |

The **Quantities** of the amplification reactions of **P190** and **ABL** for duplicates of each **sample** (Results > Report) are analysed as described in the following table that shows the different cases that might occur in an amplification session and the recommended approach to assess the data:

| Sample | mRNA of P190 | mRNA of ABL* | Calculated Quantity of mRNA of P190 | Calculated Quantity of mRNA of ABL |
|---|---|---|---|---|
| 1st replicate | DETECTED | Quantity ≥ 10,000 | Sum Quantity | Sum Quantity |
| 2nd replicate | DETECTED | Quantity ≥ 10,000 | | |
| 1st replicate | NOT DETECTED | Quantity ≥ 10,000 | 0 | Sum Quantity |
| 2nd replicate | NOT DETECTED | Quantity ≥ 10,000 | | |
| 1st replicate | Quantity < 10 copies | Quantity ≥ 10,000 | Quantity | Sum Quantity |
| 2nd replicate | NOT DETECTED | Quantity ≥ 10,000 | | |
| 1st replicate | Quantity > 10 copies | Quantity ≥ 10,000 | Retest the sample | |
| 2nd replicate | NOT DETECTED | Quantity ≥ 10,000 | | |
| 1st replicate | DETECTED or NOT DETECTED | Quantity < 10,000 | Retest the sample | |
| 2nd replicate | DETECTED or NOT DETECTED | Quantity ≥ 10,000 | | |
| 1st replicate | DETECTED or NOT DETECTED | Quantity < 10,000 | Retest the sample | |
| 2nd replicate | DETECTED or NOT DETECTED | Quantity < 10,000 | | |

*** Note:** If for a sample the result of the **ABL** amplification reactions is **ABL Quantity < 10,000** or **ABL NOT DETECTED**, this means that ABL mRNA was not detected efficiently. In this case, problems have occurred during the extraction phase (loss of RNA, presence of inhibitors or degradation of extracted RNA, see Troubleshooting) which may cause incorrect and false negative results.

**Note:** If for a sample the result of the amplification reactions is **P190 NOT DETECTED** and **ABL Quantity < 10,000** or **ABL NOT DETECTED** for at least one of two replicates, the results of assay is invalid and the sample is not suitable. The test must be repeated on extracted RNA first and, if the problem is confirmed, starting from the extraction of a new sample.

**Note:** If for a sample the result of the amplification reactions is **P190 DETECTED** and **ABL Quantity < 10,000** or **ABL NOT DETECTED** for at least one of two replicates, the results of assay is valid and the sample is positive for P190 mRNA. In this case, however, it is not possible to carry out the quantitative analysis. The test must be repeated on extracted RNA first and, if the problem is confirmed, starting from the extraction of a new sample.

**Note:** If for a sample the result of the amplification reaction is **P190 NOT DETECTED** and **ABL Quantity ≥ 10,000** for both replicates, the P190 mRNA has not been detected in the RNA obtained from the sample but it is not possible to exclude the presence of P190 mRNA at a lower titre than the detection limit of the product (see Performance Characteristics). In this case the result would be a false negative.

**Note:** If for a sample the result of the amplification reaction is **P190 Quantity > 10 copies** for one replicate and **P190 NOT DETECTED** for the other replicate and **ABL Quantity ≥ 10,000** for both replicates, the P190 mRNA has not been correctly detected in the RNA obtained from the sample. The results of assay is valid and the sample is positive for P190 mRNA. In this case, however, it is not possible to carry out the quantitative analysis. The test must be repeated on extracted RNA first and, if the problem is confirmed, starting from the extraction of a new sample.



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When the result of the amplification reactions of a sample is **P190 DETECTED** and **ABL Quantity ≥ 10,000**, the result of assay is valid, the sample is positive for P190 mRNA and it is possible to carry out the quantitative analysis.

The **calculated Quantities of P190 and ABL mRNA** of each sample are used to calculate the percentage of copies of P190 mRNA normalized to ABL mRNA copies (**P190 %**) in the starting sample according to this formula:

$$\text{P190 \%} = \frac{\text{Calculated Quantity of mRNA of P190}}{\text{Calculated Quantity of mRNA of ABL}} \times 100$$

The results obtained with this assay must be interpreted in consideration with all the clinical data and the other laboratory tests regarding the patient.

## PERFORMANCE CHARACTERISTICS

### Limit of Detection

The P190 Limit of Detection of the assay with total RNA was determined using a panel of dilutions prepared from reference calibrated material IVS-0032 Clonal Control RNA (InVivoScribe, US). The panel consists of total RNA extracted from a human cell line positive for BCR-ABL P190 e1a2 diluted in total RNA from a human cell line negative for the translocation. The dilutions used ranged from $10^{-3.5}$ to $10^{-6.0}$ (0.5 Log dilution steps). Each sample of the panel was tested in 24 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the 7500 Fast Dx Real-Time PCR Instrument. The statistical analysis was performed by Probit regression. The limit of detection was defined as the dilution at which the probability of obtaining a positive result is equal to 95%.

The final results are summed up in the following table.

| Detection limit with total RNA samples | | | |
|---|---|---|---|
| | | Confidence interval of 95% | |
| | | Lower limit | Upper limit |
| 95% positivity | $10^{-4.46}$ dilution | $10^{-4.63}$ dilution | $10^{-4.15}$ dilution |

The detection limit was defined at a dilution of $10^{-4.46}$ corresponding to a concentration of P190% between 0.0005% ($10^{-4,5}$ dilution) e 0.0081% ($10^{-4.0}$ dilution). The average amount of ABL recorded in the tests for the detection limit definition was approximately 200,000 copies per reaction.

### Linear measuring range

The P190 linear measuring range of this assay with total RNA was determined using the panel of reference calibrated material IVS-0032 Clonal Control RNA (InVivoScribe, US). The panel consists of total RNA extracted from a human cell line positive for BCR-ABL P190 e1a2 diluted in total RNA from a human cell line negative for the translocation. The dilutions used ranged from pure P190 positive RNA (P190 RNA) to $10^{-6.0}$ (1 Log dilution steps). Each sample of the panel was tested in 24 replicates (300 ng of RNA / reaction), carrying out the reverse transcription and amplification reaction by ELITechGroup S.p.A. products in association with the 7500 Fast Dx Real-Time PCR Instrument. The statistical analysis was performed by linear regression.

The analysis of the data obtained demonstrated that the assay has a linear response for the panel points from pure P190 positive RNA to $10^{-4}$ with a linear correlation coefficient greater than 0.99.

The upper limit of the linear measurement verified in this test is the pure P190 positive RNA, corresponding to a concentration of P190% equal to 69.9%.

The lower limit of the linear measurement verified in this test is the dilution $10^{-4.0}$, equal to the Limit of Detection and corresponding to a concentration of P190% equal to a 0.015%.

The final results are summed up in the following table.

| Linear measuring range with total RNA samples | | | |
|---|---|---|---|
| **Sample** | **Mean P190 copies / reaction** | **Mean Log P190copies /reaction** | **Std Dev** |
| P190 RNA | 226,290.72 | 5.35 | 0.07 |
| $10^{-1.0}$ dilution | 28,245.34 | 4.45 | 0.07 |
| $10^{-2.0}$ dilution | 3,715.29 | 3.57 | 0.06 |
| $10^{-3.0}$ dilution | 535.35 | 2.72 | 0.10 |
| $10^{-4.0}$ dilution | 26.44 | 1.38 | 0.19 |

The mean quantity of ABL recorded in the tests for the definition of the linear measuring range was approximately 250,000 copies per reaction.

The measured quantity of ABL were verified by using the European certified reference material ERM®-AD623 (IRMM, Belgium). The material consists of a dilution panel (1.0 Log dilution steps) of plasmid DNA containing ABL amplification products. The plasmid DNA concentration was calculated by digital PCR method. The dilutions used ranged from $10^6$ copies / µL to $10^1$ copies / µL. Each sample of the panel was tested in 9 replicates carrying out the amplification reaction by ELITechGroup S.p.A. products «BCR-ABL P190 ELITe MGB® Kit» and «BCR-ABL P190 ELITe Standard» in association with the 7500 Fast Dx Real-Time PCR Instrument.

The data analysis, performed according to IRMM recommendations, showed that measured values of certified reference material obtained with ELITechGroup S.p.A. products are within the measurement uncertainty for quantities from $10^6$ copies / µL to $10^1$ copies / µL (equivalent to 10,000,000 copies per reaction and to 100 copies per reaction, by using 10 µL per reaction) and therefore they are aligned with European certified reference material ERM®-AD623 (IRMM, Belgium).

The final results are summed up in the following table.

| Alignment of ABL measurement to the European reference material ERM®-AD623 | | |
|---|---|---|
| **Certified Copies / µL** | **Measured Copies / µL** | **Standard Deviation** |
| 1,108,000 | 1,121,250 | 86,262 |
| 108,000 | 111,797 | 14,429 |
| 10,300 | 12,769 | 2,119 |
| 1,020 | 1,314 | 261 |
| 104 | 146 | 31 |
| 10 | 16 | 3 |

**Detection and quantification efficiency on possible polymorphisms**

The analytical sensitivity of the assay, as the efficiency of detection and quantification with possible polymorphisms, was evaluated by comparison of sequences with nucleotide databases.

The verification of the hybridization regions of the primer oligonucleotides and of fluorescent probes (P190 and ABL) by alignment with the sequences of P190 and ABL human genes available in database showed their preservation and the absence of significant mutations.

**Diagnostic sensitivity: confirmation of positive samples**

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested analyzing a panel of P190 positive clinical samples.

The diagnostic sensitivity was evaluated using 45 archived RNA samples extracted from lymphomonocyte and leukocyte suspensions from leukemia patients tested positive for P190 with a real time amplification product. The samples were extracted with a method validated in the reference laboratory. The total extracted RNA (300 ng / reaction) reverse transcription and amplification reactions were carried out with ELITechGroup S.p.A. products on 7500 Fast Dx Real Time PCR Instrument.



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The final results are summed up in the following table.

| Samples | N | positive | negative |
|---|---|---|---|
| P190 Positive RNA from lymphomonocyte and leukocyte suspensions | 45 | 41 | 4 |

Forty-one samples were confirmed positive (P190 detected), with a medium amount of ABL of about 115,000 copies / reaction. When tested by the reference product, the four discordant negative samples have a very low titre (< 3 copies per reaction) and just one out of two replicates was positive.

In this test the diagnostic sensitivity of the assay was equal to 91.1%.

**Diagnostic specificity: confirmation of negative samples**

The diagnostic specificity of the assay, as confirmation of negative samples, was tested analysing a panel of P190 negative clinical samples.

The diagnostic specificity was evaluated using 52 archived RNA samples extracted from lymphomonocyte and leukocyte suspensions obtained from patients tested negative for P190 by a commercial CE IVD molecular diagnostic product. The samples were extracted with a method validated in the reference laboratory. The total extracted RNA (300 ng / reaction) reverse transcription and amplification reactions were carried out with ELITechGroup S.p.A. products on 7500 Fast Dx Real Time PCR Instrument.

The final results are summed up in the following table.

| Samples | N. | positive | negative |
|---|---|---|---|
| P190 negative RNA from lymphomonocyte and leukocyte suspensions | 52 | 12 | 40 |

In the first instance forty samples were confirmed negative from the qualitative point of view (P190 not detected), with a medium amount of ABL of about 91,000 copies / reaction. The twelve discordant positive samples presented a quantity of P190 extremely low (around 1 copy / reaction and only in one of the duplicates). The literature reports the detection at low titer, similar to minimal residual disease, of translocation t(9;22) variant P190 in peripheral blood samples of healthy individuals (Biernaux C. et. al. and Bose S. et. al.).

Taking into account these evidences, it is possible to state the diagnostic specificity of the assay was equal to 100%.

**Note:** The complete data and results of the tests carried out to evaluate the performance characteristics of the product with matrices and instruments are recorded in the Product Technical File " BCR-ABL P190 ELITe MGB® Kit", FTP RTSG07PLD190.

## REFERENCES

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C. Biernaux et. al. (1995) *Blood* 86: 3118 - 3122
S. Bose et al. (1998) *Blood* 92: 3362 -3 367
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## PROCEDURE LIMITATIONS

Only use RNA extracted with this product from the following clinical samples: suspensions of lymphomonocytes or leukocytes from peripheral blood collected in EDTA or citrate, bone marrow blood collected in EDTA or citrate.

Do not use RNA extracted from heparinized samples: heparin inhibits the reverse transcription and amplification reactions of nucleic acids and causes invalid results.

Do not use RNA contaminated by haemoglobin, dextran, Ficoll® ethanol, or 2-propanol: these substances may inhibit the reverse transcription reaction and amplification reactions of the nucleic acids and cause invalid results.

Quantities of RNA more than 1.5 µg per reaction could inhibit the reverse transcription reaction and amplification reactions of the nucleic acids.

Do not use RNA with high quantities of human genomic DNA that can inhibit the reverse transcription and amplification reactions of nucleic acids and cause invalid results.

There is no data available concerning inhibition caused by antibiotics, antiviral drugs, chemotherapeutic drugs or immunosuppressants.

The results obtained with this product are subject to the correct identification, collection, transport, storage and preparation of samples. To avoid incorrect results it is therefore necessary to take particular care during these phases and to carefully follow the instructions provided with the products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real time amplification assay of nucleic acids used in this product is subject to contamination from clinical samples that are positive for P190, from positive controls and from the amplification reaction products themselves. Contamination leads to false positive results. The product has been designed in such a way as to reduce contamination; nevertheless, this phenomenon can only be prevented by following good laboratory practices and by complying scrupulously with the instructions provided in this manual.

This product must be handled by personnel qualified and trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of work clothes and premises that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product must be handled by personnel qualified and trained in molecular biology techniques, such as extraction, reverse transcription and amplification and detection of nucleic acids, to avoid incorrect results.

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.

This product requires the use of special clothing and instruments for extraction/preparation of amplification reactions and for amplification/detection of amplification products to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A negative result obtained with this product means that the P190 mRNA is not detected in the reverse transcription reaction from the RNA extracted by the sample; but it can not be excluded that the P190 mRNA has a lower titre than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

Results obtained with this product may sometimes be invalid due to inefficient detection of ABL mRNA and require retesting, starting from extraction, that can lead to a delay in obtaining final results.

Possible polymorphisms within the regions of the patient's genome covered by the product primers and probes may impair detection and quantification of P190 mRNA and ABL mRNA.

As with any diagnostic device, the results obtained with this product must be interpreted in consideration with of all the clinical data and other laboratory tests undertaken on the patient.

As with any diagnostic device, there is a residual risk of obtaining invalid results, false positives and false negatives with this product. This residual risk cannot be eliminated or reduced any further. In particular situations, this residual risk can contribute to incorrect decisions with potentially grave consequences for the patient.



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## TROUBLESHOOTING

| **Target not detected in the Q - PCR Standard reactions or in the Positive control or invalid determination coefficient of the Standard curve** | |
|---|---|
| **Possible Causes** | **Solutions** |
| Incorrect complete reaction mix preparation | Check the reagent volumes dispensed during complete reaction mix preparation |
| Incorrect dispensing into the microplate wells. | Take care when dispensing reagents into the microplate wells and comply with the work sheet.<br>Check the volumes of complete reaction mix dispensed.<br>Check the volumes of standard dispensed. |
| Incorrect session setup on ELITe InGenius | Check the position of reaction mixture, positive control or standards.<br>Check the volumes of reaction mixture, positive control or standards. |
| Probe degradation. | Use a new aliquot of PreMix. |
| PCR MasterMix degradation. | Use a new aliquot of PCR MasterMix. |
| Positive control or Standard degradation. | Use a new aliquot of standard or positive control. |
| Instrument setting error. | Check the position settings for the standard reactions on the instrument.<br>Check the thermal cycle settings on the instrument. |
| Instrument error. | Contact ELITechGroup Technical Service. |

| **Target detected in the Negative control reaction** | |
|---|---|
| **Possible Causes** | **Solutions** |
| Incorrect dispensing into the microplate wells. | Avoid spilling the contents of the sample test tube.<br>Always change tips between one sample and another.<br>Take care when dispensing samples, negative control and standards into the microplate wells and comply with the work sheet. |
| Incorrect session setup on ELITe InGenius | Check the position of reaction mixture or negative control.<br>Check the volumes of reaction mixture or negative control. |
| Error while setting the instrument. | Check the position settings of the samples, negative control and standards on the instrument. |
| Microplate badly sealed. | Take care when sealing the microplate. |
| Contamination of the molecular biology grade water. | Use a new aliquot of water. |
| Contamination of the complete reaction mix. | Prepare a new aliquot of complete reaction mix. |
| Contamination of the extraction / preparation area for amplification reactions. | Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use. |
| Instrument error. | Contact ELITechGroup Technical Service. |

| **Unexpected amplification profile of the target or target not detected in the sample reaction** | |
|---|---|
| **Possible Causes** | **Solutions** |
| Incorrect complete reaction mix preparation. | Check the reagent volumes dispensed during complete reaction mix preparation; verify that RT EnzymeMix was added to complete reaction mix. |
| Incorrect dispensing into the microplate wells. | Avoid spilling the contents of the sample test tube.<br>Always change tips between one sample and another.<br>Take care when dispensing samples into the microplate wells and comply with the work sheet. |
| Incorrect session setup on ELITe InGenius | Check the position of reaction mixture or samples.<br>Check the volumes of reaction mixture or samples. |
| Inhibition due to sample interfering substances. | Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session.<br>Repeat the extraction and amplification of sample, performing a further washing step of the white cells pellet, to remove all red cells before lysis.. |
| RT EnzymeMix degradation. | Use a new aliquot of RT EnzymeMix. |
| Problems during reagent storage. | Verify that RT EnzymeMix was not exposed to temperatures higher than -20°C longer than 10 minutes.<br>Verify that the complete complete reaction mix was not exposed to room temperature longer than 30 minutes. |
| Problems during extraction | Verify quality and concentration of extracted RNA. |
| Instrument error. | Contact ELITechGroup Technical Service. |

| **Irregular or high background fluorescence in the reactions** | |
|---|---|
| **Possible causes** | **Solutions** |
| Incorrect dispensing of sample. | Carefully mix by pipetting 3 times when adding samples, negative control and standards into the complete reaction mixture. Avoid creating bubbles both at the well bottom and in surface. |
| Baseline setting error. | Set the baseline calculation range the within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g. from cycle 6 to cycle 15.<br>Use the automatic baseline calculation by setting the "Auto Baseline" option. |

| **Error 30103 on ELITe InGenius** | |
|---|---|
| **Possible causes** | **Solutions** |
| Too high concentration of target in the sample. | If significant amplification is observed in PCR plot:<br>- repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or<br>- repeat the extraction with a 1:10 dilution in molecular biology grade water of sample in an “Extract + PCR” session. |



**BCR-ABL P190 ELITe MGB® Kit**
reagents for RNA reverse transcription and cDNA Real Time amplification

REF RTSG07PLD190

## SYMBOLS

REF — Catalogue Number.

Upper limit of temperature.

LOT — Batch code.

Use by (last day of month).

IVD — *in vitro* diagnostic medical device.

Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.

Contains sufficient for "N" tests.

Attention, consult instructions for use.

CONT — Contents.

Keep away from sunlight.

Manufacturer.

## NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Life Technologies Corporation and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Life Technologies Corporation. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008. Phone: +1(760)603-7200. Fax: +1(760)602-6500. Email: outlicensing@thermofisher.com.

ELITe® MGB detection reagents are covered by one or more of U.S. Patent numbers 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers 0819133, 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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# BCR-ABL P190 ELITe MGB® kit used with ELITe InGenius®

Code: RTSG07PLD190

**This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com**
**This document is available only in English.**

## A. Intended use

The «BCR-ABL P190 ELITe MGB® Kit» product is a qualitative and quantitative, reverse transcription and amplification of nucleic acids assay for the detection of the mRNA of the BCR-ABL rearrangement, t(9;22) translocation, Philadelphia chromosome, variant P190 (P190) and for the quantification of the mRNA of P190 compared with the mRNA of the gene codifying the kinase protein Abelson (ABL). The assay is CE-IVD validated in combination with the instrument **ELITe InGenius®**.

## B. Amplified sequence

| | Gene | Fluorophore |
|---|---|---|
| **Target** | BCR-ABL (variant P190 e1a2) | FAM |
| **Control** | ABL (exons a2a3) | FAM |

## C. Validated matrix

› **PBL isolated by buffycoat***

*Lympho-monocyte and/or leukocyte suspensions must be extracted from buffy coat from Peripheral Blood matrix

## D. Kit content

| P190 PreMix | ABL PreMix | PCR Master Mix | RT Enzyme Mix* |
|---|---|---|---|
|  |  |  |  |
| 1 tube of 270 µL<br>36 reactions<br>6 freeze-thaw cycles<br>**PURPLE CAP** | 1 tube of 270 µL<br>36 reactions<br>6 freeze-thaw cycles<br>**NEUTRAL CAP** | 2 tubes of 820 µL<br>36 reactions<br>6 freeze-thaw cycles per tube<br>**NEUTRAL CAP** | 2 tubes of 20 µL<br>36 reactions<br>6 freeze-thaw cycles per tube<br>**BLACK CAP** |

› Maximum shelf-life: 18 months

› 18 determinations in duplicate

› Storage Temperature: -20°C

** The RT EnzymeMix must not be exposed to temperatures higher than -20 °C for more than 10 minutes*

## E. Material required not provided in the kit

› **ELITe InGenius instrument:** INT030
› **ELITe InGenius SP RNA:** INT034SPRNA
› **ELITe InGenius DNase I:** INT034DNASE
› **Dnase Tube Adapter Kit:** G6431-000
› **Cell Lysis Solution Promega*:** A7933
› **RNA Lysis Buffer Promega*:** Z3051
› **Thioglycerol Promega*:** A208B-C
** or equivalent*

› **ELITe InGenius PCR Cassette:** INT035PCR
› **ELITe InGenius SP200 Consumable Set:** INT032CS
› **BCR-ABL P190 - ELITe Positive Control:** CTRG07PLD190
› **BCR-ABL P190 ELITe Standard:** STDG07PLD190
› **ELITe InGenius Waste Box:** F2102-000
› **300 µL Filter Tips Axygen:** TF-350-L-R-S
› **2 mL Sarstedt tube** :72.694.005

## F. ELITe InGenius protocol

| | |
|---|---|
| › Sample volume | 200 µL |
| › Total eluate volume | 100 µL |
| › PCR eluate input volume | 10 µL for each PCR mix |
| › BCR-ABL Q-PCR Mix volume | 20 µL for each PCR mix |
| › Report unitage | %P190 |
| Frequency of controls | 15 days |
| › Frequency of calibration | 60 days |

## G. Sample pre-treatment

The sample need a blood pre-treatment to separate leukocyte by buffy-coat isolation, according to laboratory use or referring the indications shown in the “Samples and Controls” paragraph of the instruction for use.

## H. Procedure

For the Calibration follow the table below:

| Target | Number of Samples | PreMix | PCR MasterMix | RT EnzymeMix |
|---|---|---|---|---|
| P190 | 5 | 30 µL | 90 µL | 0.9 µL |
| ABL | 3 | 20 µL | 60 µL | 0.6 µL |

For Controls and samples follow the table below:

| Number of Samples | P190 or ABL PreMix | PCR MasterMix | RT Enzyme Mix |
|---|---|---|---|
| 1 | 15 µL | 45 µL | 0.5 µL |
| 2 | 25 µL | 75 µL | 0.8 µL |
| 3 | 40 µL | 120 µL | 1.2 µL |

The complete reaction mixtures should be used within 5 hours when kept on board in the refrigerated block. This time allows to carry out 1 working session of 3.5 hours and to start a second working session. It's important to mix them between the runs. The complete reaction mixtures **cannot be stored**.

## I. Performance

| Matrix | Limit of Detection | Diagnostic Sensitivity | Diagnostic Specificity |
|---|---|---|---|
| (PBL) Peripheral Blood Leukocyte | **0.0032%** | **100%** 24/24* | **96.7%** 29/30* |

**confirmed samples/ tested samples*

## J. Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, reverse-transcription, amplification and result interpretation are automatically performed. Three operational modes are available: complete run, or extraction only, or PCR only.

### Before analysis

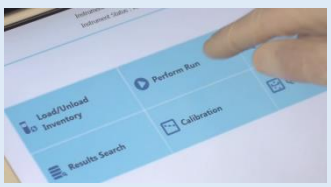
**1.** Switch on ELITe InGenius
Identification with username and password
Select the mode "Closed" or "Open"

Verify calibrators: BCR-ABL P190 Q-PCR Standard in the "Calibration menu". Verify controls: BCR-ABL P190 pos. and neg. controls in the "Control menu"
NB: Both have been run, approved and not expired

**2.** Thaw all the reagents and prepare 2 complete reaction mixture (P190 and ABL Mix) by adding into the dedicated 2 mL tube the calculated volumes of the three components for each Mix.
Mix by vortexing at low speed for 10 seconds three times, centrifuge the tube for 5 seconds
The complete reaction mixture should be used within **5** hours when kept on board in the refrigerated block

### Procedure 1 - Complete run: Extraction + PCR

**1.** Select "Perform Run" on the touch screen



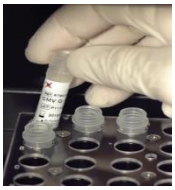

**2.** Verify the extraction volumes.
Input: "200 µL", eluate: "100 µL"


**3.** Scan the sample barcodes with hand-held barcode reader or type the sample ID

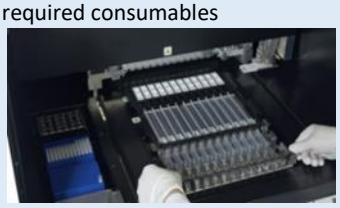
**4.** Select the “Assay protocol BCR-ABL P190 ELITe_PBL_200_100”

**5.** Select the sample position: sonication tube

**6.** Load the complete reaction mixture on the “Inventory Block”

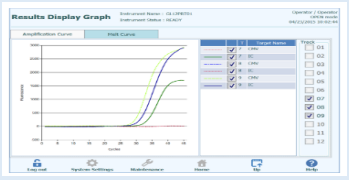


**7.** Load: PCR cassette, the ELITe InGenius SP RNA extraction cartridges, the ELITe InGenius DNase I and all the required consumables



**8.** Close the door
Start the run


**9.** View, approve and store the results




## Procedure 2 - PCR only

**1 to 4**: Follow the Complete Run procedure described above

**5.** Select the protocol “PCR only” and set the sample position “Elution tube”

**6.** Load the extracted nucleic acid tubes in the rack n°4

**7.** Load the PCR cassette rack
Load the complete reaction mixture in the inventory block

**8.** Close the door
Start the run

**9.** View, approve and store the results

## Procedure 3 - Extraction only

**1 to 4**: Follow the Complete Run procedure described above

**5.** Select the protocol “Extraction Only” and set the sample position: sonication tube

**6.** Load: the ELITe InGenius SP RNA extraction cartridges, the ELITe InGenius DNase I and all the required consumables

**7.** Close the door
Start the run

**8.** Archive the eluate sample

# BCR-ABL P190 ELITe MGB® Kit used with ABI PCR instrument

Code: RTSG07PLD190

ELITechGroup
EMPOWERING IVD

**This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com**
**This document is available only in English.**

## A. Intended use

**The BCR-ABL P190 ELITe MGB Kit** is a qualitative and quantitative, reverse transcription and amplification of nucleic acids assay for the detection of the mRNA of the BCR-ABL rearrangement, t(9;22) translocation, *Philadelphia* chromosome, variant P190 (P190) and for the quantification of the mRNA of P190 compared with the mRNA of the gene codifying the kinase protein Abelson (ABL). The assay is CE-IVD validated in combination with ABI PCR thermal cyclers (Thermo-Fisher) and laboratory validated extraction system such as the «Maxwell® CSC» (Promega) automatic extraction system or other equivalent products.

### Amplified sequence

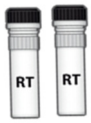
| | Gene | Fluorophore |
|---|---|---|
| **Target** | **BCR-ABL rearrangement (variant P190 e1a2)** | FAM |
| **Internal Control** | **ABL (exons a2a3)** | FAM |

## B. Validated matrix

› **Peripheral blood collected in EDTA or sodium citrate or bone marrow ***

› *Lympho-monocyte and/or leukocyte suspensions must be extracted from matrices mentioned above.

## C. Kit content

| P190 PreMix | ABL PreMix | PCR Master Mix | RT Enzyme Mix* |
|---|---|---|---|
|  |  |  |  |
| 1 tube of 270 µL<br>50 reactions<br>6 freeze-thaw cycles<br>**PURPLE CAP** | 1 tube of 270 µL<br>50 reactions<br>6 freeze-thaw cycles<br>**NEUTRAL CAP** | 2 tubes of 820 µL<br>50 reactions<br>6 freeze-thaw cycles per tube<br>**NEUTRAL CAP** | 2 tubes of 20 µL<br>50 reactions<br>6 freeze-thaw cycles per tube<br>**CAP with BLACK INSERT** |

› Maximum shelf-life: 18 months
25 reactions in duplicate

› Storage Temperature: -20°C

** The RT Enzyme Mix must not be exposed to temperatures higher than -20 °C for more than 10 minutes*

## D. Material required not provided in the kit

› **Maxwell® CSC:** AS6000
› **7500 Fast Dx, 7300 and 7900 PCR Instrument**
› **BCR-ABL P190 ELITe Standard:** STDG07PLD190
› **BCR-ABL P190 - ELITe Positive Control:** CTRG07PLD190
› **Molecular biology grade water**

## E. Performance

| System | Matrix | Limit of Detection | Diagnostic Sensitivity | Diagnostic Specificity |
|---|---|---|---|---|
| Maxwell - ABI | Peripheral blood or bone marrow | **0,0015% P190%**<br>$10^{-4.5}$ Dilution | **91.1%** (41/45)* | **100%** (40/40)* |

**confirmed samples/ tested samples*

## F. Procedure

The procedure below summarizes the main steps of the sample analysis with conventional PCR workflow: validated extraction systems, PCR instrument settings, PCR set-up and result interpretation.

### Complete reaction mixtures reconstitution

› Thaw P190 PreMix and ABL PreMIx, PCR MasterMix, vortex 10 sec three times, spin down 5 sec
› RT Enzyme Mix should not be exposed to T° > -20°C more than 10min. Gently shake, spin down 5 sec
› Prepare two 1.5 ml tube, one for the complete reaction mixture of P190 and the other for complete reaction mixture ABL
› Calculate the required volume of the 3 components for each complete reaction mixture

**Note:** the volumes indicated in the table are sufficient for the set up of the reactions for reverse transcription and real time amplification required for the number of samples to be tested, negative control and four Q-PCR Standards, in duplicate plus an adequate safety margin.

| Samples | PreMix | PCR MasterMix | RT Enzyme Mix |
|---|---|---|---|
| 1 | 65 µL | 195 µL | 3.9 µL |
| 2 | 75 µL | 225 µL | 4.5 µL |
| 3 | 85 µL | 255 µL | 5.1 µL |
| 4 | 95 µL | 285 µL | 5.7 µL |
| 5 | 110 µL | 330 µL | 6.6 µL |
| 6 | 120 µL | 360 µL | 7.2 µL |
| 7 | 130 µL | 390 µL | 7.8 µL |
| 8 | 140 µL | 420 µL | 8.4 µL |
| 9 | 150 µL | 450 µL | 9.0 µL |
| 10 | 160 µL | 480 µL | 9.6 µL |
| 11 | 170 µL | 510 µL | 10.2 µL |
| 12 | 180 µL | 540 µL | 10.8 µL |
| 13 | 190 µL | 570 µL | 11.4 µL |
| 14 | 205 µL | 615 µL | 12.3 µL |
| 15 | 215 µL | 645 µL | 12.9 µL |
| 16 | 225 µL | 675 µL | 13.5 µL |
| 17 | 235 µL | 705 µL | 14.1 µL |
| 18 | 245 µL | 735 µL | 14.7 µL |
| 19 | 255 µL | 765 µL | 15.3 µL |

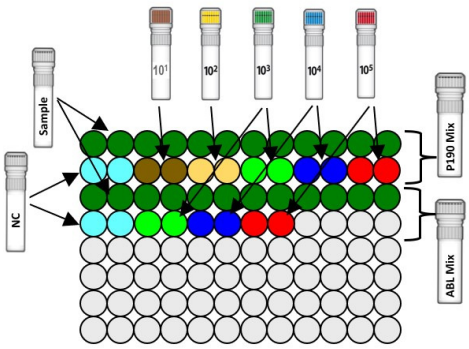
### Amplification - Settings of 7500 Fast Dx and 7300, 7900 PCR instruments

1. Switch on the thermal-cycler
2. Set “P190” detector with “FAM” and quencher “none”
3. Set “ABL” detector with “FAM” and quencher “none”
4. Set passive reference as “Cy5” with 7500 Fast Dx and as “ROX” with 7300, 7900 instruments
5. Set up the thermal profile as indicated. Fluorescence acquisition must be set during hybridization step at 56°C

| Stage | Temperature | Timing |
|---|---|---|
| Reverse Transcription | 50°C | 20 min |
| Initial Denaturation | 94°C | 5 min |
| Amplification and detection<br>*45 cycles* | 94°C<br>56°C<br>72°C | 10 sec<br>30 sec<br>15 sec |

### Amplification - PCR Set-up

1. Thaw BCR-ABL P190 Q-PCR standard tubes
2. Mix gently and spin-down
3. Prepare the “P190” and “ABL” complete reaction mixtures by adding the required volume of three components as reported in table above. The complete reaction mixture should be used within 30 min and cannot be stored
4. Pipet **20 µL** of “P190” complete reaction mixture after reconstitution in the microplate wells in use
5. Pipet **20 µL** of “ABL” complete reaction mixture after reconstitution in the microplate wells in use
6. Add, **10 µL** of extracted RNA in sample wells, **10 µL** of molecular grade water in Negative Control well, and **10 µL** of the 5 Q-PCR Standards in standard curve wells
7. Extracted RNA samples, Q-PCR Standards and Negative Control must be pipetted in duplicate
8. Seal the microplate with the amplification sealing sheet
9. Transfer the microplate in the thermocycler and start



## Amplification - Threshold for quantitative analysis

| Instrument | P190 FAM | ABL FAM |
|---|---|---|
| 7500 Fast Dx Real Time PCR | 0.1 | 0.1 |
| 7300 and 7900 Real Time PCR | 0.1 | 0.1 |

## Interpretation - quantitative results

| Detector FAM | mRNA | Quantity of mRNA |
|---|---|---|
| Ct determinated | Detected | Quantity |
| **Ct Undetermined** | **Not detected** | **0** |

| Sample | mRNA of P190 | mRNA of ABL | Calculated Quantity of mRNA of P190 | Calculated Quantity of mRNA of ABL |
|---|---|---|---|---|
| **1st replicate** | **DETECTED** | **Quantity ≥ 10,000** | **Sum Quantity** | **Sum Quantity** |
| **2nd replicate** | **DETECTED** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **NOT DETECTED** | **Quantity ≥ 10,000** | **0** | **Sum Quantity** |
| **2nd replicate** | **NOT DETECTED** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **Quantity < 10 copies** | **Quantity ≥ 10,000** | **Quantity** | **Sum Quantity** |
| **2nd replicate** | **NOT DETECTED** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **Quantity > 10 copies** | **Quantity ≥ 10,000** | **Retest the sample** | |
| **2nd replicate** | **NOT DETECTED** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **DETECTED or NOT DETECTED** | **Quantity < 10,000** | **Retest the sample** | |
| **2nd replicate** | **DETECTED or NOT DETECTED** | **Quantity ≥ 10,000** | | |
| **1st replicate** | **DETECTED or NOT DETECTED** | **Quantity < 10,000** | **Retest the sample** | |
| **2nd replicate** | **DETECTED or NOT DETECTED** | **Quantity < 10,000** | | |

## Percentage of copies of P190 mRNA normalized to ABL mRNA copies (P190 %)

| Detector FAM | mRNA | P190 % |
|---|---|---|
| P190 Ct determinated | Detected | $\frac{\text{Calculated Quantity of mRNA of P190}}{\text{Calculated Quantity of mRNA of ABL}} \times 100$ |
| ABL Ct determinated | Detected (**Quantity ≥ 10,000**) | |