Instructions for use

BCR-ABL Dx ELITe MGB® Kit

reagents for RNA reverse transcription and Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF RTSG07ING

UDI 08033891487485

CE 0123

IVD

CHANGE HISTORY

Revision	Notice of change	Date (dd/mm/yyyy)
01	New product development	26/07/2024

TABLE OF CONTENT

1 INTENDED USE

The product **BCR-ABL Dx ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids reverse transcription and Real-Time PCR assay for the detection of the mRNA of the *BCR:: ABL* (BCR-ABL) rearrangement, and the discrimination of the main variants extracted from clinical specimens.

The assay is able to detect and identify **p190 e1a2**, **p195 e6a2**, **p200 e8a2**, **p210 e13a2** and **e14a2** (typing by melting analysis), **p230 e19a2** in the first reaction and **p190 e1a3**, **p195 e6a3**, **p200 e8a3**, **p210 e13a3** and **e14a3** (typing by melting analysis), **p230 e19a3** in the second reaction.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, reverse transcription, Real-Time PCR and results interpretation, using human specimens of peripheral blood leukocyte (PBL).

The product is intended for use as an aid in the diagnosis of BCR::ABL positive leukemia in patients suspected of having a leukemia linked to BCR::ABL rearrangement.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

2 ASSAY PRINCIPLE

The assay is a qualitative multiplex One-Step Reverse Transcription Real-Time PCR detecting and identifying the main BCR-ABL isoforms mRNAs in total RNA isolated from PBL specimens and then retro- transcribed and amplified in two reactions using **BCR-ABL a2** and **BCR-ABL a3** complete reaction mixtures, that contain primers and probes with ELITe MGB technology.

Table 1

Detected BCR-ABL isoform mRNAs		
	BCR-ABL a2 PCR Mix	**BCR-ABL a3 PCR Mix**
p190	**e1a2**	**e1a3**
p195	**e6a2**	**e6a3**
p200	**e8a2**	**e8a3**
p210 (typing by melting analysis)	**e13a2**	**e13a3**
	e14a2	**e14a3**
p230	**e19a2**	**e19a3**

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct) and the melting temperatures (Tm).

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

3 PRODUCT DESCRIPTION

The **BCR-ABL Dx ELITe MGB Kit** provides the following components:

- **BCR-ABL a2 PCR Mix,** an optimized and stabilized PCR mixture that contains the specific primers and probes for:
  - p190 e1a2 mRNA, detected in Channel **p190a2**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher® and labelled with FAM dye,

- p195 e6a2 mRNA, detected in Channel **p195a2**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor ® 593 (AP593) dye,
- p200 e8a2 mRNA, detected in Channel **p200a2**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 559 (AP559) dye,
- p210 e13a2 and e14a2 mRNAs, detected in Channel **p210a2**; the probes are stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 639 (AP639) dye,
- p230 e19a2 mRNA, detected in Channel **p230a2**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 690 (AP690) dye,
- ABL mRNA, as endogenous Internal Control, detected in Channel **ICa2**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 525 (AP525) dye,

The **BCR-ABL a2 PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

- **BCR-ABL a3 PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:
  - p190 e1a3 mRNA, detected in Channel **p190a3**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with FAM dye,
  - p195 e6a3 mRNA, detected in Channel **p195a3**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 593 (AP593) dye,
  - p200 e8a3 mRNA, detected in Channel **p200a3**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 559 (AP559) dye,
  - p210 e13a3 and e14a3 mRNAs, detected in Channel **p210a3**; the probes are stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 639 (AP639) dye,
  - p230 e19a3 mRNA, detected in Channel **p230a3**; the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 690 (AP690) dye, stabilized by the MGB and quenched by the EDQ,
  - ABL mRNA, as endogenous Internal Control, detected in Channel **ICa3**, the probe is stabilized by the MGB, quenched by the Eclipse Dark Quencher and labelled with AquaPhluor 525 (AP525) dye.

  The **BCR-ABL a3 PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.
- **RT EnzymeMix,** an optimized and stabilized mixture of enzymes for reverse transcription.

The **BCR-ABL Dx ELITe MGB Kit** contains sufficient reagents for **48 tests** on the **ELITe InGenius** and **ELITe BeGenius**, with 20 µL of PCR Mixes and 0.3 µL RT EnzymeMix used per reaction.

The **BCR-ABL Dx ELITe MGB Kit** can be also used in association with equivalent instruments.

4 MATERIALS PROVIDED IN THE PRODUCT

Table 2

Component	Description	Quantity	Classification of hazards
BCR-ABL a2 PCR Mix ref. RTSG07INGA2	Mixture of reagents for reverse transcription and Real-Time PCR in tube with **WHITE cap**	**2 x 600 µL**	**-**
BCR-ABL a3 PCR Mix ref. RTSG07INGA3	Mixture of reagents for reverse transcription and Real-Time PCR in tube with **YELLOW cap**	**2 x 600 µL**	**-**
RT EnzymeMix ref. RTS003-RT	Reverse transcription enzymes in tube with **cap with BLACK insert**	**2 x 20 µL**	**-**

5 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~5,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- 50 mL tube with screwcap (Sarstedt, Germany, ref. 62.547.254).
- 15 mL tube with screwcap (Sarstedt, Germany, ref. 62.554.502).
- 2.0 mL skirted tube with screwcap (Sarstedt, ref. 72.694.005).
- Molecular biology grade water.

6 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample, the amplification positive and negative controls and the consumables are **not** provided with this product.

For automated extraction of nucleic acids, reverse transcription, Real-Time PCR and result interpretation of samples, the following products are required.

Table 3

Instruments and softwares	Products and reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA, ref. INT030). **ELITe InGenius Software** version 1.3.0.19 (or later). **BCR-ABL a2 ELITe _PC** **BCR-ABL a3 ELITe _PC** Assay Protocols with parameters for Positive Controls analysis **BCR-ABL a2 ELITe _NC** **BCR-ABL a3 ELITe _NC** Assay Protocols with parameters for Negative Controls analysis **BCR-ABL a2 ELITe _PBL_200_100** **BCR-ABL a3 ELITe _PBL_200_100** Assay Protocols with parameters for PBL specimen analysis.	**ELITe InGenius SP RNA** (EG SpA, ref. INT034SPRNA). **ELITe InGenius DNase I** (EG SpA. INT034DNASE). **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS). **Alternative Caps For Extraction Tubes** (EG SpA, ref. 925-CAP) with ELITe InGenius only (optional). **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR). **ELITe InGenius DNase tube adapter Kit** (EG S.p.A. ref. G6431-000). **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000). **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only. **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only. **BCR-ABL Dx - ELITe Positive Control** (EG SpA, ref. CTRG07ING). **Cell Lysis Solution** (Promega, code A7933 or equivalent reagent). **RNA Lysis Buffer** (Promega, code Z3051 or equivalent reagent). **Thioglycerol** (Promega, code A208B-C or equivalent reagent).
ELITe BeGenius (EG SpA, ref. INT040). **ELITe BeGenius Software** version 2.2.1 (or later). **BCR-ABL a2 ELITe _Be_PC** **BCR-ABL a3 ELITe _Be_PC** Assay Protocols with parameters for Positive Controls analysis **BCR-ABL a2 ELITe _Be_NC** **BCR-ABL a3 ELITe _Be_NC** Assay Protocols with parameters for Negative Controls analysis **BCR-ABL a2 ELITe _Be_PBL_200_100** **BCR-ABL a3 ELITe _Be_PBL_200_100** Assay Protocols with parameters for PBL specimen analysis.	

7 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

7.1 General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) 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 infectious. 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 neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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 the 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 the instructions provided before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

Only use reagents provided with the product and those recommended by the manufacturer.

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

7.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR 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.

Laboratory coats, gloves and tools dedicated to work session setup are needed.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. 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 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.

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

The PCR Cassette must be handled carefully and never opened to avoid PCR product diffusion into the environment and sample and reagent contamination.

7.3 Warnings and precautions specific for the components

Table 4

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles
BCR-ABL a2 PCR Mix	-20 °C or below (protected from light)	one month	up to five
BCR-ABL a3 PCR Mix			
RT EnzymeMix	-20 °C or below	one month	up to ten times, for up to ten minutes at +2 / +8 °C

8 SPECIMENS AND CONTROLS

8.1 Specimens

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified and handled according to laboratory guidelines, and collected, transported, and stored under the following conditions:

Table 5

Specimen	Collection requirements	Transport/Storage conditions
		+16 / +26 °C (room temperature)
Peripheral blood	collected in EDTA or sodium citrate	within 24 hours and no later than 48 hours

NOTE

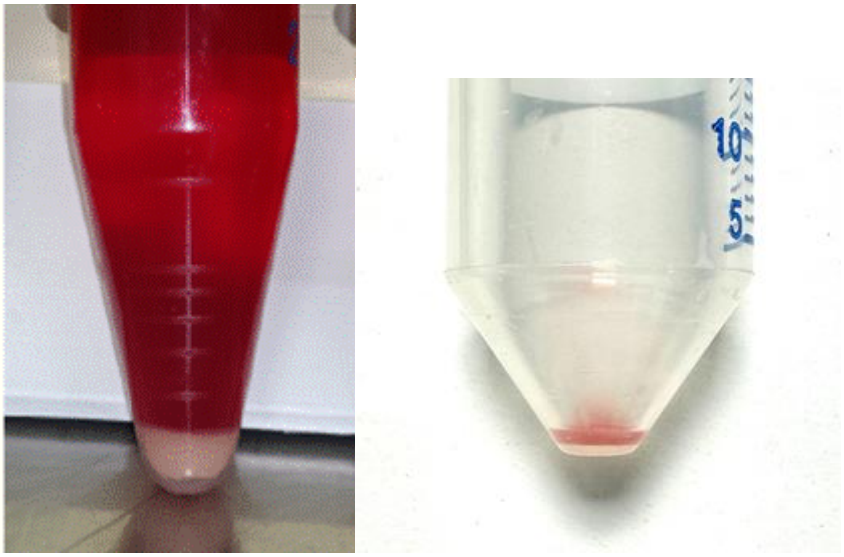
Do not freeze peripheral blood in order to prevent degradation of RNA.

When starting with peripheral blood, it is mandatory to separate leukocytes according to the following indications.

Table 6

	A. Pre-treatment procedure for leukocyte isolation with Buffy Coat	B. Pre-treatment procedure for leukocyte isolation with Direct Lysis
1	Prepare 15 mL tubes and 2 mL tubes needed and label them with a permanent marker.	Prepare 50 mL tubes and 2 mL tubes needed and label them with a permanent marker.
2	Not applicable	Dispense **Cell Lysis Solution** (Promega, ref. A7933) into a 50 mL tube: use **15 mL** if starting from 5 mL of blood or **30 mL** if starting from 10 mL of blood (3:1 ratio).
3	Mix peripheral blood samples collected in EDTA or sodium citrate thoroughly by inversion.	
4	Transfer **5 - 10 mL of fresh peripheral blood** into the 15 mL tube.	Transfer **5 - 10 mL of fresh peripheral blood** into the 50 mL tube.
5	Centrifuge for **10 minutes at 3,000 RCF** (with no brake applied).	Not applicable
6	Dispense **5 mL of Cell Lysis Solution** (Promega, ref. A7933) into a new 15 mL tube.	Not applicable
7	With a 1 mL pipette, **remove the buffy coat** obtained after centrifugation and transfer it into the 15 mL tube containing the Cell Lysis Solution. Wash the tip in the solution until it is free of cells.	Not applicable

Table 6 (continued)

	A. Pre-treatment procedure for leukocyte isolation with Buffy Coat	B. Pre-treatment procedure for leukocyte isolation with Direct Lysis
8	Incubate at **room temperature for 10 minutes** mixing by inversion (no vortex) at least 3-4 times.	
9	Centrifuge for **10 minutes at 3,000 RCF**.	
10	**NOTE** The ideal quantity of white cells is represented, in 1:1 scale, in the following picture.  • If the pellet is equal to or smaller than the one shown above, remove the supernatant, resuspend the pellet in **1.5 mL of Cell Lysis Solution** and transfer it into a 2.0 mL tube. • If the pellet is bigger than the one shown above, remove the supernatant, resuspend the pellet in **3 mL of Cell Lysis Solution** and transfer 1.5 mL into two different 2.0 mL tubes.	
11	Centrifuge again for about **2 minutes at 3,000 RCF**.	
12	Carefully **remove the supernatant** (attention to remove traces of red cells above the white cells pellet).	
13	Carefully lyse the pellet in **200 µL of Homogenization Solution** (1 mL of RNA Lysis Buffer, Promega, ref. Z3051 + 20 µL of 1-Thioglycerol, Promega, Ref. A208B-C) by pipetting.	

The PBL lysate can be immediately used or stored under the following conditions:

Table 7

Specimen type	Buffer requirements	Storage conditions	
		-20 ± 10 °C	**-70 ± 15 °C**
PBL lysate	Homogenization Solution	≤ one month	≤ one month

To perform samples testing on the **ELITe InGenius** and **ELITe BeGenius**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 8

Assay Protocols for BCR-ABL Dx ELITe MGB Kit				
Specimen	**Instrument**	**Assay Protocol Name**	**Report**	**Characteristics**
PBL	**ELITe InGenius**	**BCR-ABL a2 ELITe_PBL_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
		BCR-ABL a3 ELITe_PBL_200_100		
	ELITe BeGenius	**BCR-ABL a2 ELITe_Be_PBL_200_100**		
		BCR-ABL a3 ELITe_Be_PBL_200_100		

For all protocols, 200 µL of **pre-treated sample** must be used:

- with **ELITe InGenius**, transfer the PBL lysate from the 2.0 mL tube to an **Extraction tube** (avoid producing bubbles during pipetting),
- with **ELITe BeGenius**, the 2.0 mL tube containing the PBL lysate is directly used

NOTE

Before loading the pre-treated samples on instruments, spin down the content for 5 seconds and keep on ice or cool block. When **Extraction tube** is used, it is recommended also to use the dedicated cap (SpA, ref. 925-CAP).

NOTE

Pipetting samples to the **Extraction tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the “Warnings and Precautions” section.

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to “Potentially Interfering Substances” in the Performance Characteristics section to check data concerning interfering substances.

8.2 PCR controls

PCR control results must be generated and approved for each lot of PCR reagent.

- For the Positive Control, use the **BCR-ABL a2 Positive Control** and **BCR-ABL a3 Positive Control**, components of the product **BCR-ABL Dx - ELITe Positive Control** (not provided with this kit), with respectively the **BCR-ABL a2 ELITe_PC** or **BCR-ABL a2 ELITe_Be_PC** and **BCR-ABL a3 ELITe_PC** or **BCR-ABL a3 ELITe_Be_PC** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **BCR-ABL a2 ELITe_NC** or **BCR-ABL a2 ELITe_Be_NC** and **BCR-ABL a3 ELITe_NC** or **BCR-ABL a3 ELITe_Be_NC** Assay Protocols.

NOTE

- The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the PCR control validation for each lot of PCR reagent. PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and negative controls. The PCR controls must be re-run if any of the following events occur:
- a new lot of reagents is used,
- results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius** or **ELITe BeGenius**.

8.3 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

9 ELITe InGenius PROCEDURE

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

Table 9

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	A) Validation of Positive Control and Negative Control results
		B) Validation of sample results
		C) Sample result reporting

9.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Controls" menu on the Home page, verify the PCR Controls (**BCR-ABL a2 Positive Control, BCR-ABL a2 Negative Control, BCR-ABL a3 Positive Control, BCR-ABL a3 Negative Control**) are approved and valid (Status) for the **PCR Mix** lot (**BCR-ABL a2 PCR Mix** and **BCR-ABL a3 PCR Mix**) to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- 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 EG SpA (see "Specimens and Controls").

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

9.2 STEP 2 - Session Setup

The **BCR-ABL Dx ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

With the product **BCR-ABL Dx ELITe MGB Kit**, in both setup A or B, two reactions should be performed for each sample: one with **BCR-ABL a2 PCR Mix** (for BCR-ABL a2 isoforms) and the other with **BCR-ABL a3 PCR Mix** (for BCR-ABL a3 isoforms).A maximum of 6 samples can be analyzed in each session on **ELITe InGenius.**

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

1. Thaw the needed **BCR-ABL a2 PCR Mix** tubes (WHITE cap) and **BCR-ABL a3 PCR Mix** tubes (YELLOW cap) at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session).Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **48 tests**. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

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

3. Prepare one 2 mL tube (Sarstedt, ref. 72.694.005, not included in the kit) for each **complete reaction mixture** and label it with a permanent marker.
4. Calculate the needed volumes of **BCR-ABL a2 PCR Mix, BCR-ABL a3 PCR Mix** and **RT EnzymeMix** for preparing the **complete reaction mixtures** on the basis of the number of samples (N) to be analyzed, as described in the table below.

Table 10

Samples Number (N)	BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Prepare the **complete reaction mixtures** by transferring into the labeled 2 mL tube the calculated volumes of the two components. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

The **complete reaction mixtures** can be used within 7 hours if kept refrigerated (for 2 sessions of 3.5 hours each). The complete reaction mixture and **cannot** be stored for re-use

NOTE

The **complete reaction mixtures** are sensitive to the light, do not expose them to direct light.

6. Starting from PBL lysates **(setup A)**, for each sample, perform an integrated run (“Extract + PCR” mode) with **BCR-ABL a2 PCR Mix** and an amplification run (“PCR Only” mode) with **BCR-ABL a3 PCR Mix**.
7. Starting from extraction products **(setup B)**, for each sample, perform two amplification runs (“PCR Only” mode) with both **BCR-ABL a2 PCR Mix** and **BCR-ABL a3 PCR Mix**.

NOTE

It is advisable to create a template on the instrument to facilitate the setup of the session (See ELITe InGenius manual).

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 11

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify pre-treated samples** and, if needed, thaw them at room temperature. Mix gently, spin down the content for 5 seconds and keep on ice or cool block. For this assay, **200 µL of sample** must be transferred in an Extraction tube previously labelled.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Controls** tubes (a2 and a3) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 4 reactions.
2	Not applicable	Not applicable	**Prepare** the **Negative Controls** (a2 and a3) by transferring at least 50 µL of molecular biology grade water into an “Elution tube”, provided with ELITe InGenius SP 200 Consumable Set.
3	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen.
4	Ensure the “ Extraction Input Volume” is 200 µL and the “ Extracted Elute Volume” is 100 µL.	Ensure the ”Extraction Input Volume” is 200 µL and the “ Extracted Elute Volume” is 100 µL.	Ensure the "Extraction Input Volume” is 200 µL and the "Extracted Elute Volume” is 100 µL.
5	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	Not applicable
6	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”): for each sample assign a2 Assay Protocol and a3 Assay Protocol in the next Track.	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”): for each eluate assign a2 Assay Protocol and a3 Assay Protocol in the next Track.	**Select the Assay Protocols** (a2 and a3, see “Specimens and Controls”) in the “Assay” column. Enter lot number and expiry date for Positive Control and Negative Control (molecular biology grade water).
7	For **a2 Assay Protocol**: ensure the “Protocol” displayed is “Extract + PCR” and select the “Sample Position” as “Extraction Tube”.	For **a2 Assay Protocol**: select “PCR Only” in the “Protocol” column and the sample loading position in the “Sample Position” column as “Elution Tube (bottom row)”.	Ensure “PCR Only” is selected in the “Protocol” column.
8	For **a3 Assay Protocol**: assign the “Protocol” “PCR Only” and select as “Sample Position” the Track of the sample.	For **a3 Assay Protocol**: select “PCR Only” in the “Protocol” column and the sample loading position in the “Sample Position” column as the Track of the eluate.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.
9	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
10	**Load** the **complete reaction mixtures** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **complete reaction mixtures** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **complete reaction mixtures** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.
11	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
12	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
13	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
14	**Load** PCR Cassette, ELITe InGenius SP RNA extraction cartridges, ELITe InGenius DNase I tubes (uncapped) and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tube with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
15	In the DNase I screen, Insert “DNase I” as “Reagent Name”, lot number and expiry date.	Not applicable	Not applicable
16	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
17	Close the instrument door.	Close the instrument door.	Close the instrument door.
18	Press “Start”.	Press “Start”.	Press “Start”.

NOTE

It is advisable to wait a few minutes after the start of the run, to check the correct aspiration of the samples from the extraction tube. Occasionally, the instrument may give Error 20081 (see section “Troubleshooting”).

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results, 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 ± 10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

The **complete reaction mixtures** can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3.5 hours each).Mix gently, then spin down the contents for 5 seconds before starting next session. The complete reaction mixture cannot be stored for re-use.

NOTE

At the end of the run, the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Control. The remaining Negative Control must be discarded.

NOTE

The **BCR-ABL a2 Positive Control** and **BCR-ABL a3 Positive Control** can be used for 4 separate sessions of 3.5 hours each.

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

9.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen, the results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”).Refer to the instrument manual for more details.

NOTE

When **BCR-ABL Dx ELITe MGB Kit** is used, two amplification runs for each sample are performed using **BCR-ABL a2 PCR Mix** (for BCR-ABL a2 isoforms) and **BCR-ABL a3 PCR Mix** (for BCR-ABL a3 isoforms).The results analysis shall be executed for both runs.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **BCR-ABL Dx ELITe MGB Kit** through the following procedure:

A. Validation of Positive Control and Negative Control results,

B. Validation of sample results,

C. Sample result reporting.

9.3.1 A. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the targets of the Positive Control and Negative Control reactions with the **BCR-ABL a2 ELITe_PC**, **BCR-ABL a3 ELITe_PC**, **BCR-ABL a2 ELITe_NC** and **BCR-ABL a3 ELITe_NC** Assay Protocols parameters. The resulting Ct and Tm values are used to verify the system (reagents lot and instrument).

The Positive Control and Negative Control results, specific for the PCR reagent lot, are recorded in the database (Controls).They can be viewed and approved by “Administrator” or “Analyst” users, following the GUI instructions.

The Positive Control and Negative Control results expire **after 15 days**.

The results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

9.3.2 B. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (Channels **p190a2**, **p195a2**, **p200a2**, **p210a2**, **p230a2**) and the Internal Control (Channel **ICa2**) with the **BCR-ABL a2 ELITe_PBL_200_100** Assay Protocol parameters and the targets (Channels **p190a3**, **p195a3**, **p200a3**, **p210a3**, **p230a3**) and the Internal Control (Channel **ICa3**) with the **BCR-ABL a3 ELITe_PBL_200_100** Assay Protocol parameters.

Results are shown in “Results Display” screen.

The sample results can be approved when the conditions in the tables below are true.

Table 12

Assay with BCR-ABL a2 PCR Mix	
1) Positive Control	**Status**
BCR-ABL a2 Positive Control	APPROVED
2) Negative Control	**Status**
BCR-ABL a2 Negative Control	APPROVED

Table 13

Assay with BCR-ABL a3 PCR Mix	
1) Positive Control	**Status**
BCR-ABL a3 Positive Control	APPROVED
2) Negative Control	**Status**
BCR-ABL a3 Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius software** using Assay Protocol parameters. The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the RNAs are either detected or not detected.

Table 14

Assay with BCR-ABL a2 PCR Mix	
Result of sample run	**Interpretation**
p190a2:RNA Detected e1a2	**p190 mRNA was detected** in the sample. The isoform is **e1a2**.
p190a2:RNA Detected Typing not determined	**p190 mRNA was detected** but the Tm analysis was not possible.
p190a2:RNA Not detected or below the LoD	**p190 mRNA was not detected** in the sample. The sample is negative for p190 mRNA, or its concentration is below the Limit of Detection of the assay.
p200a2:RNA Detected e8a2	**p200 mRNA was detected** in the sample. The isoform is **e8a2**.
p200a2:RNA Detected Typing not determined	**p200 mRNA was detected** but the Tm analysis was not possible.
p200a2:RNA Not detected or below the LoD	**p200 mRNA was not detected** in the sample. The sample is negative for p200 mRNA, or its concentration is below the Limit of Detection of the assay.
p195a2:RNA Detected e6a2	**p195 mRNA was detected** in the sample. The isoform is **e6a2**.
p195a2:RNA Detected Typing not determined	**p195 mRNA was detected** but the Tm analysis was not possible.
p195a2:RNA Not detected or below the LoD	**p195 mRNA was not detected** in the sample. The sample is negative for p195 mRNA, or its concentration is below the Limit of Detection of the assay.
p210a2:RNA Detected e13a2	**p210 mRNA was detected** in the sample. The isoform is **e13a2**.
p210a2:RNA Detected e14a2	**p210 mRNA was detected** in the sample. The isoform is **e14a2**.

Table 14 (continued)

Assay with BCR-ABL a2 PCR Mix	
Result of sample run	**Interpretation**
p210a2:RNA Detected e13a2 e14a2	**p210 mRNA was detected** in the sample. Both the isoforms, **e13a2** and **e14a2**, are present.
p210a2:RNA Detected Typing not determined	**p210 mRNA was detected** but the Tm analysis was not possible.
p210a2:RNA Not detected or below the LoD	**p210 mRNA was not detected** in the sample. The sample is negative for p210 mRNA, or its concentration is below the Limit of Detection of the assay.
p230a2:RNA Detected e19a2	**p230 mRNA was detected** in the sample. The isoform is **e19a2**.
p230a2:RNA Detected Typing not determined	**p230 mRNA was detected** but the Tm analysis was not possible.
p230a2:RNA Not detected or below the LoD	**p230 mRNA was not detected** in the sample. The sample is negative for p230 mRNA, or its concentration is below the Limit of Detection of the assay.
Invalid - Retest Sample.	**Not valid assay result** caused by Internal Control failure. The test should be repeated.

Table 15

Assay with BCR-ABL a3 PCR Mix	
Result of sample run	**Interpretation**
p190a3:RNA Detected e1a3	**p190 mRNA was detected** in the sample. The isoform is **e1a3**.
p190a3:RNA Detected Typing not determined	**p190 mRNA was detected** but the Tm analysis was not possible.
p190a3:RNA Not detected or below the LoD	**p190 mRNA was not detected** in the sample. The sample is negative for p190 mRNA, or its concentration is below the Limit of Detection of the assay.
p200a3:RNA Detected e8a3	**p200 mRNA was detected** in the sample. The isoform is **e8a3**.
p200a3:RNA Detected Typing not determined	**p200 mRNA was detected** but the Tm analysis was not possible.
p200a3:RNA Not detected or below the LoD	**p200 mRNA was not detected** in the sample. The sample is negative for p200 mRNA, or its concentration is below the Limit of Detection of the assay.
p195a3:RNA Detected e6a3	**p195 mRNA was detected** in the sample. The isoform is **e6a3**.
p195a3:RNA Detected Typing not determined	**p195 mRNA was detected** but the Tm analysis was not possible.
p195a3:RNA Not detected or below the LoD	**p195 mRNA was not detected** in the sample. The sample is negative for p195 mRNA, or its concentration is below the Limit of Detection of the assay.
p210a3:RNA Detected e13a3	**p210 mRNA was detected** in the sample. The isoform is **e13a3**.
p210a3:RNA Detected e14a3	**p210 mRNA was detected** in the sample. The isoform is **e14a3**.
p210a3:RNA Detected e13a3 e14a3	**p210 mRNA was detected** in the sample. Both the isoforms, **e13a3** and **e14a3**, are present.
p210a3:RNA Detected Typing not determined	**p210 mRNA was detected** but the Tm analysis was not possible.

Table 15 (continued)

Assay with BCR-ABL a3 PCR Mix	
Result of sample run	**Interpretation**
p210a3:RNA Not detected or below the LoD	**p210 mRNA was not detected** in the sample. The sample is negative for p210 mRNA, or its concentration is below the Limit of Detection of the assay.
p230a3:RNA Detected e19a3	**p230 mRNA was detected** in the sample. The isoform is **e19a3**.
p230a3:RNA Detected Typing not determined	**p230 mRNA was detected** but the Tm analysis was not possible.
p230a3:RNA Not detected or below the LoD	**p230 mRNA was not detected** in the sample. The sample is negative for p230 mRNA, or its concentration is below the Limit of Detection of the assay.
Invalid-Retest Sample.	**Not valid assay result** caused by Internal Control failure. The test should be repeated.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control RNA was not efficiently detected, which could be due to problems in sample collection, pretreatment, extraction, reverse transcription or PCR steps (e.g., incorrect sampling, degradation or loss of RNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see “Troubleshooting”).

Samples reported as “**pXXXxx: RNA Detected Typing not determined**” are not suitable for melting analysis. In this case, a Ct was detected but the Tm was not detected or outside the established range, due to problems in sample collection, pretreatment, extraction, reverse transcription or PCR steps (e. g., incorrect sampling, degradation or loss of RNA during the extraction, inhibitor carry-over in the eluate or high background), which may cause incorrect results.

If sufficient eluate volume remains, it is suggested to retest the sample (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is the same, the sample should be retested starting from extraction of a new sample using “Extract + PCR” mode (see “Troubleshooting”).

Samples reported as “**pXXXxx: RNA: Not detected or below the LoD**” are suitable for analysis but BCR-ABL RNA was not detected. In this case, the sample may be either negative for BCR-ABL RNA or the BCR-ABL RNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics”).

NOTE

In case of a positive test with multiple isoforms, verify the Ct value of each variant. The co-expression of the additional BCR-ABL isoforms should only be diagnosed when their Ct values are comparable to the main isoform detected at the lowest Ct (see N. P. C. Cross et al. 2023). Otherwise, only the main isoform at the lowest Ct value should be reported.

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

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

9.3.3 C. Sample result reporting

The sample results are stored in the database and reports can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the results details by selected sample (SID).

The “Track Report” shows the results details by selected Track.

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

10 ELITe BeGenius PROCEDURE

The procedure to use the **BCR-ABL Dx ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 16

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only),
		C) Positive Control and Negative Control run (PCR Only).
STEP 3	Review and approval of results	A) Validation of Positive Control and Negative Control results
		B) Validation of sample results
		C) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in “**CLOSED**” mode,
- in the “Controls” menu on the Home page, verify the PCR Controls (**BCR-ABL a2 Positive Control**, **BCR-ABL a2 Negative Control**, **BCR-ABL a3 Positive Control**, **BCR-ABL a3 Negative Control**) are approved and valid (Status) for the **PCR Mix** lot (**BCR-ABL a2 PCR Mix** and **BCR-ABL a3 PCR Mix**) to be used. If no valid PCR Controls are available for the **PCR Mix** lot, run the PCR Controls as described in the following sections,
- 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 EG SpA (see “Specimens and Controls”).

If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

10.2 STEP 2 - Session Setup

The **BCR-ABL Dx ELITe MGB Kit** can be used on **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

With the product **BCR-ABL Dx ELITe MGB Kit**, in both setup A or B, two PCR should be performed for each extracted sample: one with **BCR-ABL a2 PCR Mix** (for BCR-ABL a2 isoforms) and the other with **BCR-ABL a3 PCR Mix** (for BCR-ABL a3 isoforms). A Maximum of 12 samples can be analyzed in each session on **ELITe BeGenius.**

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

1. Thaw the needed **BCR-ABL a2 PCR Mix** tubes (WHITE cap) and **BCR-ABL a3 PCR Mix** tubes (YELLOW cap) at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

2. Take the needed **RT EnzymeMix** tubes. Each tube is sufficient for **48 tests**. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

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

3. Prepare one 2 mL tube (Sarstedt, ref. 72.694.005, not included in the kit) for each **complete reaction mixture** and label it with a permanent marker.
4. Calculate the needed volumes of **BCR-ABL a2 PCR Mix**, **BCR-ABL a3 PCR Mix**, and **RT EnzymeMix** for preparing the **complete reaction mixture** on the basis of the number of samples (N) to be analyzed, as described in the table below.

Table 17

Sample Number (N)	BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL
13 ≤ N ≤ 18	(N + 3) x 20 µL	(N + 3) x 0.3 µL
19 ≤ N ≤ 23	(N + 4) x 20 µL	(N + 4) x 0.3 µL
N = 24	580 µL	8.7 µL

5. Prepare the **complete reaction mixture** by transferring in the labeled 2 mL tube the calculated volumes of the two components. Mix by vortexing at low speed for 10 seconds three times, then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

The **complete reaction mixture** can be used within **7** hours if kept refrigerated (for 2 sessions of 3.5 hours each).The complete reaction mixture **cannot** be stored for re-use.

NOTE

The **complete reaction mixture** is sensitive to the light, do not expose it to direct light.

6. Starting from PBL lysates (**setup A**), for each sample perform an integrated run (“Extract + PCR” mode) using **BCR-ABL a2 PCR Mix** and an amplification run (“PCR Only” mode) using **BCR-ABL a3 PCR Mix**.
7. Starting from extraction products (**setup B**), for each sample perform two amplification runs (“PCR Only” mode) using both **BCR-ABL a2 PCR Mix** and **BCR-ABL a3 PCR Mix**.

NOTE

It is advisable to create a template on the instrument to facilitate the setup of the session (See ELITe BeGenius manual).

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 18

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify pre-treated samples** and, if needed, thaw them at room temperature; mix gently, spin down the content for 5 seconds and keep on ice or cool block. For this assay, **200 µL of sample** must be in the 2mL Sarstedt tube.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Controls** tubes (a2 and a3) at room temperature for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Not applicable	Not applicable	**Prepare** the **Negative Controls** (a2 and a3) by transferring at least 50 µL of molecular biology grade water in an “Elution tube”, provided with ELITe InGenius SP 200 Consumable Set.
3	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen.
4	Remove all the Racks from the “Cooler Unit” and place them on the preparation table.	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) of the “Cooler Unit” and place them on the preparation table	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) from the “Cooler Unit” and place them on the preparation table.
5	Select the “Run mode”: “**Extract + PCR**”.	Select the “Run mode”: “**PCR Only**”.	Select the “Run mode”: “**PCR Only**”.
6	**Load the samples** into the “Sample Rack”. When secondary tubes “2 mL Tubes” are loaded, use the blue adaptors for the “Sample Rack”.	**Load the samples** into the “Elution Rack”.	**Load the Positive Control and Negative Control** tubes into the “Elution Rack”.
7	**Insert** the “**Sample Rack**” into the “Cooler Unit” starting from the “Lane 5” (L5). Insert the “Sample ID” (SID) for each “Position” used (If secondary tubes are loaded, flag “2 mL Tube”. If secondary tubes are not barcoded, type manually the “Sample ID”).	**Insert** the “**Elution Rack**” into the “Cooler Unit” starting from “Lane 3” (L3). For each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).	**Insert** the “**Elution Rack**” into the “Cooler Unit” starting from the “Lane 3” (L3). If needed, for each “Position” enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
8	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
9	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL.	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL.	Ensure “Extraction Input Volume” is 200 µL and “Extracted Elute Volume” is 100 µL.
10	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”): for each sample to extract assign both a2 (Extract + PCR) and a3 (PCR Only) Assay Protocols.	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”): for each eluate assign both a2 and a3 Assay Protocols.	**Select the Assay Protocol** (a2 and a3, see “Specimens and Controls”) in the “Assay” column.
11	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.

Table 18 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
12	Load the "Elution tubes" into the "Elution Rack" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
13	Insert the "Elution Rack" into the "Cooler Unit" starting from "Lane 3" (L3).	Not applicable	Not applicable
14	Click "Next" to continue.	Not applicable	Not applicable
15	**Load** the **complete reaction mixture** into the "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into the "Reagent/Elution Rack".	**Load** the **complete reaction mixture** into the "Reagent/Elution Rack".
16	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). For each PCR Mix reagent enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
17	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
18	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
19	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
20	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.	Load the "**PCR Rack**" with "PCR Cassette" in the Inventory Area.
21	Click "Next" to continue.	Click "Next" to continue.	Click "Next" to continue.
22	Load the "**Extraction Rack**" with the "ELITe InGenius SP RNA" extraction cartridges, "ELITe InGenius DNase I" tubes (uncapped) and the required extraction consumables.	Not applicable	Not applicable
23	In the DNase I screen, Insert "DNase I" as "Reagent Name", lot number and expiry date.	Not applicable	Not applicable
24	Close the instrument door.	Close the instrument door.	Close the instrument door.
25	Press "Start".	Press "Start".	Press "Start".

NOTE

It is advisable to wait a few minutes after the start of the run, to check the correct aspiration of the samples from the 2 mL Tube. Occasionally, the instrument may give Error 20081 (see section "Troubleshooting").

When the session is finished, the **ELITe BeGenius** allows users to view, approve, store the results, 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 ± 10°C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

The **complete reaction mixture** can be kept on board in the refrigerated block up to 7 hours (for 2 sessions of 3.5 hours each).Mix gently, then spin down the contents for 5 seconds before starting next session. The complete reaction mixture cannot be stored for re-use.

NOTE

At the end of the run, the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Controls. The remaining Negative Control must be discarded.

NOTE

The **BCR-ABL a2 Positive Control** and **BCR-ABL a3 Positive Control** can be used for 4 separate sessions of 3.5 hours each

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

10.3 STEP 3 - Review and approval of results

The **ELITe BeGenius** monitors target and internal control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen, the results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”).Refer to the instrument manual for more details.

NOTE

When **BCR-ABL Dx ELITe MGB Kit** is used, two amplification runs for each sample are performed using **BCR-ABL a2 PCR Mix** (for BCR-ABL a2 isoforms) and **BCR-ABL a3 PCR Mix** (for BCR-ABL a3 isoforms).The results analysis shall be executed for both runs.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **BCR-ABL Dx ELITe MGB Kit** through the following procedure:

A. Validation of Positive Control and Negative Control results,

B. Validation of sample results,

C. Sample result reporting.

NOTE

Please, refer to the same paragraph of the **ELITe InGenius Procedure** for the details.

11 PERFORMANCE CHARACTERISTICS

11.1 Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was determined for BCR-ABL p210 e14a2 on ELITe BeGenius instrument by testing BCR-ABL negative RNA reference material spiked at low concentration with p210 e14a2 positive RNA reference material (Invivoscribe).

Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call. The results are reported in the following table.

Table 19

Target	LoD		95% confidence interval limits	
			Lower limit	Upper limit
p210 e14a2	Diluition	0.0000259	0.0000148	0.0000694
	Log Diluition	-4.6	-4.8	-4.2

The p210 e14a2 mRNA in samples spiked at the LoD concentration was then quantified and resulted 8 copies / reaction (p210% = 0.0066%). The calculated LoD value was verified for the targets by testing negative mRNAs extracted from PBLs samples spiked with p190 e1a2, p195 e6a2, p210 e13a2, p210 e14a2, p200 e8a2, p230 e19a2, p190 e1a3, p195 e6a3, p210 e13a3, p210 e14a3, p200 e8a3, p230 e19a3 plasmid DNAs (GenScript) at the claimed concentration on ELITe BeGenius and ELITe InGenius. When needed, the target concentration was increased. The results are reported in the following table.

Table 20

BCR-ABL a2 PCR Mix	
Target	**LoD (copies / reaction)**
p190 e1a2	8
p195 e6a2	8
p200 e8a2	50
p210 e13a2	8
p210 e14a2	8
p230 e19a2	20
BCR-ABL a3 PCR Mix	
Target	**LoD (copies / reaction)**
p190 e1a3	8
p195 e6a3	20
p200 e8a3	50
p210 e13a3	8
p210 e14a3	20
p230 e19a3	50

The LoD values reported in the table were confirmed on both ELITe BeGenius and ELITe InGenius.

11.2 Inclusivity: Efficiency of detection on different BCR-ABL isoforms

The Inclusivity of the assay, as efficiency of detection for the main isoforms of BCR-ABL, was evaluated by in silico analysis. The analysis showed a high level of sequence conservation and absence of significant mutations. So, an efficient amplification and detection of the BCR-ABL isoforms is expected.

The Inclusivity was also verified through the analysis of BCR-ABL negative RNA reference material spiked by synthetic RNA (GenScript) for each target at low concentration. The results are reported in the following table.

Table 21

BCR-ABL a2 PCR Mix			
Target	**Isoform**	**Pos. / Rep.**	**Outcome**
p190	e1a2	4 / 4	p190:RNA Detected e1a2
p195	e6a2	4 / 4	p195:RNA Detected e6a2
p200	e8a2	4 / 4	p200:RNA Detected e8a2
p210	e13a2	4 / 4	p210:RNA Detected e13a2
	e14a2	4 / 4	p210:RNA Detected e14a2
p230	e19a2	8 / 8	p230:RNA Detected e19a2
BCR-ABL a3 PCR Mix			
Target	**Isoform**	**Pos. / Rep.**	**Outcome**
p190	e1a3	4 / 4	p190:RNA Detected e1a3
p195	e6a3	4 / 4	p195:RNA Detected e6a3
p200	e8a3	4 / 4	p200:RNA Detected e8a3
p210	e13a3	4 / 4	p210:RNA Detected e13a3
	e14a3	4 / 4	p210:RNA Detected e14a3
p230	e19a3	8 / 8	p230:RNA Detected e19a3

All samples were correctly detected by the BCR-ABL Dx ELITe ELITe MGB Kit.

11.3 Interference among targets

The potential interference among targets of the assay was evaluated by a test of co-amplification in BCR-ABL negative RNA reference material spiked by plasmid DNAs (GenScript) of the more frequent isoforms.

For each target, the lower concentration detectable in all replicates is reported in the following table.

Table 22

BCR-ABL a2 PCR Mix	
Main target at 100,000 copies / reaction	**Second target at low concentration**
p190 e1a2	p210 e13a2, 100 copies / reaction
	p210 e14a2, 100 copies / reaction
p210 e13a2	p190 e1a2, 100 copies / reaction
	p210 e14a2, 10,000 copies / reaction

Table 22 (continued)

p210 e14a2	p190 e1a2, 100 copies / reaction
	p210 e13a2, 1,000 copies / reaction
BCR-ABL a3 PCR Mix	
Main target at 100,000 copies / reaction	**Second target at low concentration**
p190 e1a3	p210 e13a3, 100 copies / reaction
	p210 e14a3, 100 copies / reaction
p210 e13a3	p190 e1a3, 100 copies / reaction
	p210 e14a3, 20,000 copies / reaction
p210 e14a3	p190 e1a3, 100 copies / reaction
	p210 e13a3, 5,000 copies / reaction

The BCR-ABL Dx ELITe ELITe MGB Kit shows a minimal interference in case of co-expression of two of the most frequent BCR-ABL isoforms. When on different channels, the second target can be detected even when it is about 1000 times less than the main target.

11.4 Potentially interfering markers: Cross-reactivity

The potential cross-reactivity with other markers that may be found in whole blood samples was evaluated for the assay by *in silico* analysis. The analysis showed no significant homology with other unintended markers (unintended human gene rearrangements, viruses, bacteria, protozoa, and fungi) and therefore, no cross-reactivity is expected.

The absence of cross-reactivity with potential interfering markers was also verified through the analysis of a panel of unintended markers (Sigma-Aldrich, Invivoscribe, ATCC, ZeptoMetrix) spiked in BCR-ABL negative RNA reference material.

The results are reported in the following table were obtained with both BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix.

Table 23

Marker	Positive / Replicates						Outcome
	p190	p200	p195	p210	p230	p190	
Human genomic DNA	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
PML-RARα	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Inv(16)	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
EBV	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
CMV	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
HHV-6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
HHV-7	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
HHV-8	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
HIV-1	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity

All potentially interfering markers tested showed no cross-reactivity using the BCR-ABL Dx ELITe MGB Kit.

11.5 Potentially interfering markers: Inhibition

The potential inhibition caused by unintended markers that may be found in whole blood samples was evaluated for the assay through the analysis of a panel of unintended markers (Sigma-Aldrich, Invivoscribe, ATCC, ZeptoMetrix) spiked with plasmid DNAs (GenScript) of all the targets in BCR-ABL negative RNA reference material.

The results reported in the following table were obtained with both BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix.

Table 24

Marker	Positive / Replicates						Outcome
	p190	p200	p195	p210 e13	p210 e14	p230	
hgDNA	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
PML-RARα	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Inv(16)	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
EBV	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
CMV	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
HHV-6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
HHV-7	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
HHV-8	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
HIV-1	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference

All potentially interfering markers tested showed no inhibition of the target amplification using the BCR-ABL Dx ELITe MGB Kit.

11.6 Potentially interfering substances: Cross-reactivity

The cross-reactivity by potentially interfering substances (endogenous and exogenous), that might be found in whole blood samples, was evaluated for the assay by analysis of a panel of substances (Sigma- Aldrich) at relevant concentration spiked in BCR-ABL negative simulated samples.

The results reported in the following table were obtained with both BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix.

Table 25

Substance	Positive / Replicates					Outcome
	p190	p200	p195	p210	p230	
Hemoglobin	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Bilirubin	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Triglycerides	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
EDTA	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Heparin	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Ganciclovir	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Abacavir sulfate	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity

Table 25 (continued)

Substance	Positive / Replicates					Outcome
	p190	p200	p195	p210	p230	
Cidofovir	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Ribavirin	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Amoxicillin + clavulanate	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Cefpodoxime	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Azithromycin	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity
Ciprofloxacin	0 / 6	0 / 6	0 / 6	0 / 6	0 / 6	No cross-reactivity

The test showed that all the tested substances do not cross-react with the targets using the BCR-ABL Dx ELITe MGB Kit.

11.7 Potentially interfering substances: Inhibition

The potential inhibition of interfering substances (endogenous and exogenous), that might be found in whole blood samples, was evaluated for the assay by analysis of a panel of substances at relevant concentration spiked with plasmid DNAs (GenScript) of all the targets in BCR-ABL negative simulated samples.

The results reported in the following table were obtained with both BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix.

Table 26

Substance	Positive / Replicates						Outcome
	p190	p200	p195	p210 e13	p210 e14	p230	
Hemoglobin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Bilirubin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Triglycerides	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
EDTA	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Heparin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Ganciclovir	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Abacavir sulfate	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Cidofovir	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Ribavirin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Amoxicillin + clavulanate	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Cefpodoxime	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Azithromycin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference
Ciprofloxacin	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	6 / 6	No interference

The test showed that the tested substances do not inhibit the targets detection using the BCR-ABL Dx ELITe MGB Kit.

11.8 Cross-contamination

The possibile Cross-contamination during analysis was evaluated for the assay by testing 60 BCR-ABL negative simulated samples and 60 replicates of BCR-ABL positive simulated samples spiked with p210 e14a2 synthetic RNA (GenScript) at a concentration of ~$1x10^{10}$ copies / mL in 5 sessions.

The results are reported in the following table.

Table 27

Samples	N	Positive	Negative	%Agreement
Positive	60	60	0	100%
Negative	60	0	60	100%

In this test with the BCR-ABL Dx ELITe MGB Kit the cross-contamination was neither detected within sessions nor between sessions.

11.9 Whole system failure rate

The Whole system failure rate for the assay was evaluated by analysing 50 BCR-ABL negative PBL samples, spiked with p190 e1a2 RNA reference material (Invivoscribe) at low concentration.

The results are reported in the following table.

Table 28

Samples	N	Positive	Negative	Whole system failure rate
PBL spiked at low concentration	50	50	0	0%

In this test with the BCR-ABL Dx ELITe MGB Kit, all the PBL samples were confirmed positive and the whole system failure rate was equal to 0%.

11.10 Repeatability

The Repeatability of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of BCR-ABL negative simulated samples and BCR-ABL positive simulated samples spiked with BCR-ABL synthetic RNAs (GenScript) as follow:

- NEG: simulated sample, negative.
- GROUP A: simulated sample, positive for p190 e1a2, p210 e14a2, p190 e1a3, p210 e14a3.
- GROUP B: simulated sample, positive for p195 e6a2, p210 e13a2, p195 e6a3, p210 e13a3.
- GROUP C: simulated sample, positive for p200 e8a2, p230 e19a2, p200 e8a3, p230 e19a3.

An example of Intra-Session Repeatability (on one day) results on ELITe BeGenius is shown in the table below.

Table 29

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	6	-	-	-	-	-	-	100%
GROUP A		6	29.19	0.38	1.31	69.2	0.23	0.33	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P200 e8a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	70.0	0.34	0.48	100%
GROUP C		6	28.73	0.46	1.58	-	-	-	100%
NEG	P195 e6a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	29.99	0.25	0.85	-	-	-	100%
GROUP C		6	-	-	-	67.5	0.42	0.63	100%
NEG	P210 e13a2	6	-	-	-	-	-	-	100%
GROUP A		6	29.51	0.62	2.12	66.2	0.16	0.25	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P210 e14a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	32.11	0.44	1.38	56.5	0.18	0.31	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P230 e19a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	30.57	0.70	2.29	71.3	0.50	0.70	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	6	-	-	-	-	-	-	100%
GROUP A		6	29.83	0.14	0.46	64.0	0.08	0.12	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P195 e6a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	29.85	0.32	1.06	-	-	-	100%
GROUP C		6	-	-	-	67.9	0.17	0.25	100%

Table 29 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P200 e8a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	67.5	0.30	0.44	100%
GROUP C		6	28.41	0.27	0.95	-	-	-	100%
NEG	P210 e13a3	6	-	-	-	-	-	-	100%
GROUP A		6	29.36	0.64	2.19	65.0	0.21	0.32	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P210 e14a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	29.81	0.53	1.79	57.0	0.20	0.35	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P230 e19a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	28.94	0.41	1.43	64.9	0.13	0.19	100%

An example of Intra-Session Repeatability (on one day) on ELITe InGenius is shown in the table below.

Table 30

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	6	-	-	-	-	-	-	100%
GROUP A		6	28.30	0.37	1.31	69.8	0.08	0.12	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P200 e8a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	29.13	0.13	0.43	70.8	0.00	0.00	100%
GROUP C		6	-	-	-	-	-	-	100%

Table 30 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P195 e6a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	28.16	0.26	0.92	68.1	0.11	0.16	100%
NEG	P210 e13a2	6	-	-	-	-	-	-	100%
GROUP A		6	29.35	0.34	1.15	67.2	0.14	0.21	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P210 e14a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	31.74	0.26	0.81	57.8	0.41	0.71	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P230 e19a2	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	24.09	0.22	0.77	72.0	0.05	0.07	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	6	-	-	-	-	-	-	100%
GROUP A		6	28.58	0.53	1.85	64.5	0.06	0.10	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P195 e6a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	28.25	0.10	0.36	68.3	0.10	0.15	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P200 e8a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	27.07	0.23	0.86	68.3	0.16	0.24	100%
NEG	P210 e13a3	6	-	-	-	-	-	-	100%
GROUP A		6	29.19	0.48	1.64	65.1	0.08	0.13	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	-	-	-	-	-	-	100%

Table 30 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P210 e14a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	29.33	0.18	0.60	57.6	0.04	0.07	100%
GROUP C		6	-	-	-	-	-	-	100%
NEG	P230 e19a3	6	-	-	-	-	-	-	100%
GROUP A		6	-	-	-	-	-	-	100%
GROUP B		6	-	-	-	-	-	-	100%
GROUP C		6	25.97	0.27	1.04	65.4	0.05	0.08	100%

An example of Inter-Session Repeatability (on two days) on ELITe BeGenius is shown in the table below.

Table 31

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	12	-	-	-	-	-	-	100%
GROUP A		12	29.38	0.47	1.81	69.2	0.19	0.27	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P200 e8a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	30.13	0.31	1.03	70.1	0.62	0.89	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P195 e6a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	28.89	0.39	1.36	67.7	0.37	0.55	100%
NEG	P210 e13a2	12	-	-	-	-	-	-	100%
GROUP A		12	29.52	0.58	1.98	66.1	0.29	0.43	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%

Table 31 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P210 e14a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	32.23	0.37	1.16	56.9	0.63	1.10	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P230 e19a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	30.44	0.53	1.74	71.5	0.44	0.62	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	12	-	-	-	-	-	-	100%
GROUP A		12	29.90	0.38	1.27	64.0	0.06	0.10	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P195 e6a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	29.57	0.43	1.44	67.8	0.39	0.58	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P200 e8a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	28.22	0.33	1.18	68.0	0.15	0.22	100%
NEG	P210 e13a3	12	-	-	-	-	-	-	100%
GROUP A		12	29.27	0.65	2.22	65.0	0.20	0.31	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P210 e14a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	29.68	0.48	1.63	57.1	0.21	0.37	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P230 e19a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	28.53	0.55	1.92	65.0	0.15	0.23	100%

An example of Inter-Session Repeatability (on two days) on ELITe InGenius is shown in the table below

Table 32

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	12	-	-	-	-	-	-	100%
GROUP A		12	28.29	0.43	1.51	69.8	0.07	0.11	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P200 e8a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	29.19	0.17	0.59	70.8	0.08	0.11	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P195 e6a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	27.86	0.47	1.69	68.2	0.12	0.18	100%
NEG	P210 e13a2	12	-	-	-	-	-	-	100%
GROUP A		12	29.36	0.35	1.20	67.1	0.07	0.10	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P210 e14a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	31.78	0.25	0.78	58.5	0.11	0.19	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P230 e19a2	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	27.85	0.31	1.11	72.0	0.08	0.11	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	12	-	-	-	-	-	-	100%
GROUP A		12	28.56	0.48	1.68	64.5	0.06	0.09	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%

Table 32 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P195 e6a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	28.56	0.37	1.30	68.3	0.07	0.11	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P200 e8a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	26.78	0.36	1.34	68.3	0.14	0.21	100%
NEG	P210 e13a3	12	-	-	-	-	-	-	100%
GROUP A		12	29.12	0.42	1.45	65.1	0.08	0.13	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P210 e14a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	29.64	0.38	1.28	57.5	0.05	0.09	100%
GROUP C		12	-	-	-	-	-	-	100%
NEG	P230 e19a3	12	-	-	-	-	-	-	100%
GROUP A		12	-	-	-	-	-	-	100%
GROUP B		12	-	-	-	-	-	-	100%
GROUP C		12	25.85	0.26	1.02	65.4	0.06	0.10	100%

In the Repeatability test, the BCR-ABL Dx ELITe MGB detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 3.19%.

11.11 Reproducibility

The Reproducibility of the assay was evaluated on ELITe BeGenius and ELITe InGenius by analysis of a panel of simulated samples of PBLs negative or spiked with a panel of BCR-ABL synthetic RNAs (GenScript) as described in the previous test.

The results of Inter-Batch Reproducibility (on six days and three lots) on ELITe BeGenius are shown in the table below.

Table 33

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	36	-	-	-	-	-	-	100%
GROUP A		36	29.08	0.42	1.44	69.2	0.21	0.30	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.62	0.57	1.91	69.9	0.59	0.84	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P195 e6a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	28.77	0.49	1.69	67.6	0.28	0.42	100%
NEG	P210 e13a2	36	-	-	-	-	-	-	100%
GROUP A		36	29.25	0.50	1.72	66.1	0.70	1.06	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P210 e14a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	31.99	0.49	1.52	56.7	0.44	0.78	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	29.67	0.76	2.55	71.4	0.36	0.51	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	36	-	-	-	-	-	-	100%
GROUP A		36	29.24	0.59	2.00	63.9	0.11	0.17	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P195 e6a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.21	0.48	1.63	67.7	0.26	0.39	100%
GROUP C		36	-	-	-	-	-	-	100%

Table 33 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P200 e8a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	28.20	0.50	1.79	67.8	0.20	0.29	100%
NEG	P210 e13a3	36	-	-	-	-	-	-	100%
GROUP A		36	28.85	0.66	2.29	65.0	0.19	0.29	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P210 e14a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.66	1.07	3.61	57.1	0.17	0.29	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	28.02	0.70	2.51	64.8	0.31	0.47	100%

The results of Inter-Batch Reproducibility (on twelve days and three lots) on ELITe InGenius are shown in the table below.

Table 34

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	36	-	-	-	-	-	-	100%
GROUP A		36	27.85	0.53	1.91	69.7	0.09	0.13	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	28.73	0.41	1.42	70.8	0.07	0.10	100%
GROUP C		36	-	-	-	-	-	-	100%

Table 34 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P195 e6a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	27.58	0.49	1.77	68.2	0.12	0.18	100%
NEG	P210 e13a2	36	-	-	-	-	-	-	100%
GROUP A		36	28.66	0.72	2.50	67.1	0.37	0.55	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P210 e14a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	31.36	0.37	1.18	58.2	0.38	0.65	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	27.28	0.53	1.94	64.8	0.31	0.47	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	36	-	-	-	-	-	-	100%
GROUP A		36	27.99	0.65	2.32	64.4	0.12	0.19	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P195 e6a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	28.22	0.40	1.43	68.4	0.08	0.12	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	26.76	0.47	1.74	68.3	0.15	0.21	100%
NEG	P210 e13a3	36	-	-	-	-	-	-	100%
GROUP A		36	28.24	1.07	3.80	65.2	0.13	0.19	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%

Table 34 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P210 e14a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.10	0.63	2.18	57.6	0.10	0.17	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	25.67	0.57	2.21	65.3	0.28	0.42	100%

The results of Inter-Instrument Reproducibility (on six days, three lots and three instruments) on ELITe BeGenius are shown in the table below.

Table 35

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	36	-	-	-	-	-	-	100%
GROUP A		36	29.19	0.39	1.35	69.3	0.22	0.32	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.56	0.47	1.58	70.1	0.49	0.70	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P195 e6a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	28.71	0.44	1.55	67.7	0.15	0.23	100%
NEG	P210 e13a2	36	-	-	-	-	-	-	100%
GROUP A		36	29.09	0.46	1.58	66.2	0.69	1.04	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%

Table 35 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P210 e14a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	31.80	0.46	1.44	56.7	0.58	1.02	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	29.03	0.43	1.50	71.5	0.21	0.30	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	36	-	-	-	-	-	-	100%
GROUP A		36	29.35	0.66	2.24	63.9	0.14	0.22	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P195 e6a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.08	0.48	1.66	67.7	0.30	0.44	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	28.10	0.36	1.29	67.7	0.21	0.31	100%
NEG	P210 e13a3	36	-	-	-	-	-	-	100%
GROUP A		36	28.36	1.25	4.42	64.9	0.23	0.35	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P210 e14a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	26.69	1.05	3.54	57.1	0.20	0.36	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	27.65	0.60	2.18	64.8	0.31	0.48	100%

The results of Inter-Instrument Reproducibility (on twelve days, three lots and three instruments) on ELITe InGenius is shown in the table below.

Table 36

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P190 e1a2	36	-	-	-	-	-	-	100%
GROUP A		36	28.02	0.39	1.40	69.8	0.13	0.19	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	28.85	0.50	1.75	70.8	0.28	0.39	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P195 e6a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	27.67	0.44	1.59	68.3	0.16	0.24	100%
NEG	P210 e13a2	36	-	-	-	-	-	-	100%
GROUP A		36	28.71	0.29	1.01	67.1	0.11	0.17	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P210 e14a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	31.52	0.41	1.30	58.1	0.40	0.68	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a2	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	27.20	0.49	1.80	72.0	0.12	0.17	100%
BCR-ABL a3 PCR Mix									
NEG	P190 e1a3	36	-	-	-	-	-	-	100%
GROUP A		36	28.24	0.28	0.98	64.4	0.16	0.24	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%

Table 36 (continued)

Sample	Target	N	Ct Mean	Ct SD	Ct %CV	Tm Mean	Tm SD	Tm %CV	%Agreement
BCR-ABL a2 PCR Mix									
NEG	P195 e6a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	28.29	0.43	1.52	68.4	0.13	0.19	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P200 e8a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	26.79	0.50	1.86	68.3	0.25	0.36	100%
NEG	P210 e13a3	36	-	-	-	-	-	-	100%
GROUP A		36	28.78	0.54	1.88	65.2	0.18	0.28	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P210 e14a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	29.32	0.54	1.85	57.6	0.08	0.14	100%
GROUP C		36	-	-	-	-	-	-	100%
NEG	P230 e19a3	36	-	-	-	-	-	-	100%
GROUP A		36	-	-	-	-	-	-	100%
GROUP B		36	-	-	-	-	-	-	100%
GROUP C		36	25.65	0.69	2.68	65.3	0.30	0.46	100%

In the Reproducibility test, the BCR-ABL Dx ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 4.42%.

11.12 Diagnostic Specificity: Confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples of PBLs, certified negative or presumably negative for each target.

As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 37

Negative PBL samples	N	Positive	Negative	% Diagnostic Specificity
p190	60	0	60	100%
p195	60	0	60	100%
p200	60	0	60	100%
p210	60	0	60	100%
p230	60	0	60	100%

All PBL samples were valid and negative for analysis.

The IC Ct cut-off value is set at 31.

11.13 Diagnostic Sensitivity: Confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with ELITe InGenius by analysing clinical samples of PBLs, certified positive for each target or spiked with reference materials.

As ELITe BeGenius has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The results are summed up in the following table.

Table 38

Positive or spiked PBL samples		N	Positive	Negative	% Diagnostic Sensitivity
p190	Positive for e1a2	1	1	0	100%
	Spiked for e1a2	25	25	0	
	Spiked for e1a3	25	25	0	
p195	Spiked for e6a2	25	25	0	100%
	Spiked for e6a3	25	25	0	
p200	Positive for e8a2	1	1	0	98%
	Spiked for e8a2	25	25	0	
	Spiked for e8a3	25	24	1	
p210	Positive for e13a2	2	2	0	100%
	Spiked for e13a2	25	25	0	
	Spiked for e13a3	25	25	0	
	Positive for e14a2	3	3	0	
	Spiked for e14a2	25	25	0	
	Spiked for e14a3	25	25	0	

Table 38 (continued)

Positive or spiked PBL samples		N	Positive	Negative	% Diagnostic Sensitivity
p230	Spiked for e19a2	25	25	0	100%
	Spiked for e19a3	25	25	0	

One sample spiked for p200 e8a2 resulted discrepant negative.

NOTE

The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File “BCR-ABL Dx ELITe MGB Kit”, FTP G07ING.

12 REFERENCES

N. C. P. Cross et al. (2023) Leukemia 37: 2150 - 2167

M. Baccarani et al. (2019) Leukemia 33: 1173 - 1183.

J. Gabert et al. (2003) Leukemia. 17: 2318 – 2357

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E. A. Lukhtanov et al. (2007) Nucleic Acids Res. 35: e30

13 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: leukocytes from peripheral blood specimens collected in EDTA or sodium citrate.

Currently there are no data available concerning product performance with other clinical samples.

Do not use this product with samples of peripheral blood collected in heparin: heparin inhibits the reverse transcription and PCR reaction of nucleic acids and causes invalid results.

Do not use with this product extracted RNA containing high quantity of human genomic DNA that may inhibit the reverse transcription reaction and the amplification of nucleic acids.

The results obtained with this product depend on proper identification, collection, transport, storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the product.

Owing to its high analytical sensitivity, the Real Time PCR method used in this product is sensitive to contamination from positive clinical samples, positive controls and PCR products. Cross-contamination cause false positive results. The product format is designed to limit cross-contamination. However, cross-contamination can only be avoided by good laboratory practices and following these instructions for use.

This product must be handled by qualified personnel 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 personal protective equipment and areas 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 requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques, such as extraction, reverse transcription, PCR and detection of nucleic acids.

It is necessary to have separate areas for the preparation of the complete reaction mixture and the extraction / amplification / detection of amplification products to prevent 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 indicates that the target RNA is not detected in the RNA extracted from the sample; however, it cannot be excluded that the target RNA has a lower titer than the product detection limit (see Performance Characteristics). In this case the result could be a false negative.

In case of co-expression of more than one isoform, the sensitivity for one target can be affected by the amplification of a second target (see Performance Characteristics).

In case of a positive test with multiple isoforms, verify the Ct value of each variant. The co-expression of the additional BCR-ABL isoforms should only be diagnosed when their Ct values are comparable to the main isoform detected at the lowest Ct (see N. P. C. Cross et al. 2023). Otherwise, only the main isoform at the lowest Ct value should be reported.

In case of p210 e14a2 expression at low title, sometimes it has been detected a second non-specific Tm, resulting in a double positivity p210 e13a2 and p210 e14a2.

Results obtained with this product may sometimes be invalid due to failure of internal control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms, insertions or deletions within the region of the target RNA targeted by the product primers and probes may impair detection of target RNA.

As with any other diagnostic medical device, the results obtained with this product must be interpreted in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid, or **erroneous results** with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

14 TROUBLESHOOTING

Table 39

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, and Positive Control. Check the volumes of complete reaction mixture, and Positive Control.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Degradation of complete reaction mixture or of its components.	Do not use the complete reaction mixture for more than 2 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
Positive Control degradation.	Do not use the Positive Control for more than 4 independent sessions (3.5 hours each in the Extraction Area or in the Cooler Unit). Use a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 40

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture and Negative Control. Check the volumes of complete reaction mixture and Negative Control.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the complete reaction mixture or of its components.	Prepare again the complete reaction mixture. Use a new aliquot of components.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit.	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 41

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture and sample. Check the volumes of complete reaction mixture and sample.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its components.	Do not use the complete reaction mixture for more than 2 consecutive sessions (7 hours in the Inventory Area). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of components.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a 1:2 dilution of the pre-treated sample in Homogenization Solution, in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 42

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the Positive Control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation.

Table 43

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: -repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session. -repeat the extraction with a 1:2 dilution of the pre-treated sample in Homogenization Solution in an “Extract + PCR” session.

Table 44

Error 20081 (There is a possibility that there is no liquid in the sonication/extraction tube).	
Possible Causes	**Solutions**
Bubbles or high viscosity of the sample.	- Check the sample in extraction tube for the required tracks. If there is no sample, load sample into extraction tube for the required track. If the sample is present click the “OK” button in the dialog window to proceed with the extraction. - If it times out without action, the run will abort. In this case, if the sample is present and the session can be restarted immediately after it has been aborted, the sample could be used for the new run. Alternatively, repeat the extraction with a new aliquot of the pre-treated sample.

Table 45

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination in preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the GUI. Follow the loading sequence indicated by the software.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Prepare again the complete reaction mixture.

15 SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE 0123	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by TÜV SÜD Product Service GmbH, Germany.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Caution, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

16 NOTICE TO THE USERS

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. At the moment of the current revision of the IFU, no serious incident or recall with impact on product performance and safety of the device has occurred.

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional. Before the notice of full functionality of Eudamed has been published, the “Summary of Safety and Performance” will be made available to the public upon request by email at emd.support@elitechgroup.com, without undue delay.

17 NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Thermo Fisher Scientific. 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, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

ELITe MGB Kit® detection reagents are covered by one or more of U.S. Patent numbers 7319022, 7348146, 7381818, 7541454, 7671218, 7718374, 7723038, 7759126, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

ELITe InGenius® and ELITe BeGenius® technologies are covered by patents and pending applications.

This limited license allows the person or entity to whom the product has been provided to use the product and data generated by the use of the product, solely for human diagnostics. Neither ELITechGroup S.p.A. nor its licensors grant any other licenses, expressed or implied for any other purposes.

Appendix A BCR-ABL Dx ELITe MGB® Kit used in association with Genius series® platforms

CAUTION

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

Intended use

The product **BCR-ABL Dx ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative multiplex nucleic acids reverse transcription and Real-Time PCR assay for the detection of the mRNA of the *BCR:: ABL* (BCR-ABL) rearrangement, and the discrimination of the main variants extracted from clinical specimens.

The assay is able to detect and identify **p190 e1a2**, **p195 e6a2**, **p200 e8a2**, **p210 e13a2** and **e14a2** (typing by melting analysis), **p230 e19a2** in the first reaction and **p190 e1a3**, **p195 e6a3**, **p200 e8a3**, **p210 e13a3** and **e14a3** (typing by melting analysis), **p230 e19a3** in the second reaction.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, reverse transcription, Real-Time PCR and results interpretation, using human specimens of peripheral blood leukocyte (PBL).

The product is intended for use as an aid in the diagnosis of BCR::ABL positive leukemia in patients suspected of having a leukemia linked to BCR::ABL rearrangement.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

Amplified sequence

BCR-ABL a2 PCR Mix			
Sequence	Gene	Fluorophore	Channel
Target 1	p190 e1a2	FAM	p190a2
Target 2	P210 e13a2 and e14a2	AP639	P210a2
Target 3	P230 e19a2	AP690	P230a2
Target 4	P200 e8a2	AP559	p200a2
Target 5	p195 e6a2	AP593	p195a2
Internal Control	ABL	AP525	ICa2
BCR-ABL a3 PCR Mix			
Sequence	**Gene**	**Fluorophore**	**Channel**
Target 1	p190 e1a3	FAM	p190a3
Target 2	p210 e13a3 and e14a3	AP639	P210a3
Target 3	p230 e19a3	AP690	P230a3
Target 4	P200 e8a3	AP559	p200a3
Target 5	p195 e6a3	AP593	p195a3
Internal Control	ABL	AP525	ICa3

Validated matrix

Peripheral blood collected in EDTA or sodium citrate, pre-treated to isolate peripheral blood leukocytes (PBL).

Kit content and related products

BCR-ABL Dx ELITe MGB Kit (RTSG07ING)			**BCR-ABL Dx - ELITe Positive Control (CTRG07ING)**	
 x 2	 x 2	 x 2	 x 3	 x 3
BCR-ABL a2 PCR Mix 2 tubes of 600 µL 24 reactions per tube 48 reactions per kit 5 freeze-thaw cycles per tube	**BCR-ABL a3 PCR Mix** 2 tubes of 600 µL 24 reactions per tube 48 reactions per kit 5 freeze-thaw cycles per tube	**RT EnzymeMix** 2 tubes of 20 µL 48 reactions per tube 96 reactions per kit 10 freeze-thaw cycles	**BCR-ABL a2 Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles	**BCR-ABL a3 Positive Control** 3 tubes of 160 µL 4 reactions per tube 12 reactions per kit 4 freeze-thaw cycles
Maximum shelf-life:	**18 months**		Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**		Storage temperature	**≤ -20°C**

Other products required not provided in the kit

› ELITe InGenius instrument: INT030. › ELITe BeGenius instrument: INT040. › ELITe InGenius SP RNA: INT034SPRNA. › ELITe InGenius DNase I: INT034DNASE. › ELITe InGenius SP200 Consumable Set: INT032CS. › ELITe InGenius PCR Cassette: INT035PCR. › ELITe InGenius Waste Box: F2102-000.	› ELITe InGenius DNase tube adapter kit: G6431-000. › 300 µL Filter Tips Axigen: TF-350-L-R-S. › 1000 µL Filter Tips Tecan: 30180118. › Alternative Caps For Extraction Tubes: 925-CAP (optional). › Cell Lysis Solution (Promega, code A7933 or equivalent reagent). › RNA Lysis Buffer (Promega, code Z3051 or equivalent reagent). › Thioglycerol (Promega, code A208B-C or equivalent reagent).

ELITe InGenius and ELITe BeGenius protocol

› Sample volume › Total elution volume	200 µL 100 µL	› Eluate PCR input volume › PCR Mix volume › Frequency of controls	10 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Target		Limit of Detection	Sensitivity	Specificity
Peripheral blood collected in EDTA or sodium citrate	p190	e1a2	8 copies / reaction	**100%** (26 / 26)	**100%** (60 / 60)
		e1a3	8 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)
	p195	e6a2	8 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)
		e6a3	20 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)
	p200	e8a2	50 copies / reaction	**100%** (26 / 26)	**100%** (60 / 60)
		e8a3	50 copies / reaction	**98%** (24 / 25)	**100%** (60 / 60)
	p210	e13a2	8 copies / reaction	**100%** (27 / 27)	**100%** (60 / 60)
		e13a3	8 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)
		e14a2	8 copies / reaction	**100%** (28 / 28)	**100%** (60 / 60)
		e14a3	20 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)
	p230	e19a2	20 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)
		e19a3	50 copies / reaction	**100%** (25 / 25)	**100%** (60 / 60)

Sample preparation

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

Sample type	Transport/Storage conditions
	+16 / +26 °C **(room temperature)**
Peripheral blood collected in EDTA or sodium citrate	within 24 hours and no later than 48 hours
Note: The specimens must be pre-treated to isolate peripheral blood leukocytes (PBL) before use according to the procedure described in the complete IFU.	

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe InGenius software to setup the run. All the steps: extraction, reverse transcription, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR) or PCR Only.

NOTE

With the product BCR-ABL Dx ELITe MGB Kit two reactions should be performed for each sample: one with BCR-ABL a2 PCR Mix (for BCR-ABL a2 isoforms) and the other with BCR-ABL a3 PCR Mix (for BCR-ABL a3 isoforms).

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **BCR-ABL a2 Positive Control / BCR-ABL a3 Positive Control** and **BCR-ABL a2 Negative Control / BCR-ABL a3 Negative Control** in the "Controls" menu. Note: All must have been run, approved and not expired.	**3.** Thaw the **BCR-ABL a2 PCR Mix / BCR-ABL a3 PCR Mix** tubes. Vortex gently. Spin down 5 sec.

4. Prepare the complete reaction mixture

Samples Number (N)	BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Vortex gently
Spin down 5 sec
Keep the complete reaction mixture in ice. Do not expose to direct light.

Procedure 1 – Complete run: Extract + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen.	**2.** Verify the extraction volumes: Input: "200 µL", elution: "100 µL".	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID.
4. Select the "Assay Protocol" of interest: BCR-ABL a2 ELITe_PBL_200_100 and BCR-ABL a3 ELITe_PBL_200_100	**5.** For a2 Assay Protocol select the method "Extract + PCR" and the sample position "Extraction Tube". For a3 Assay Protocol select the method "PCR Only" and select as sample position the Track of the sample.	**6.** Load the complete reaction mixtures in the Inventory Block.
7. Load: PCR cassette, SP RNA Extraction cartridge, DNase I tubes, Elution tube, Tip Cassette, Extraction Tube racks.	**8.** Close the door. Start the run.	**9.** View, approve and store the results.

NOTE

If an Extract Only mode is needed, refer to the instrument user's manual for procedure.

Procedure 2: PCR Only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen.	**2.** Verify the extraction volumes: Input: “200 µL”, elution: “100 µL”.	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID.
4. Select the “Assay Protocol” of interest: BCR-ABL a2 ELITe_PBL_200_100 and BCR-ABL a3 ELITe_PBL_200_100 or BCR-ABL a2 ELITe_PC or BCR-ABL a3 ELITe_PC or BCR-ABL a2 ELITe_NC or BCR-ABL a3 ELITe_NC	**5.** Select the method “PCR Only”. For controls and a2 Assay Protocol select the sample position “Elution Tube”. For a3 Assay Protocol select as sample position the Track of the eluate.	**6.** Load the complete reaction mixtures in the Inventory Block.
7. Load PCR Cassette rack and the Elution tube rack with the extracted nucleic acid.	**8.** Close the door. Start the run.	**9.** View, approve and store the results.

ELITe BeGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe BeGenius software to setup the run. All the steps: extraction, reverse transcription, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: (Extract + PCR) or PCR Only.

NOTE
With the product BCR-ABL Dx ELITe MGB Kit two reactions should be performed for each sample: one with BCR-ABL a2 PCR Mix (for BCR-ABL a2 isoforms) and the other with BCR-ABL a3 PCR Mix (for BCR-ABL a3 isoforms).

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode “**CLOSED**”.	**2.** Verify controls: **BCR-ABL a2 Positive Control / BCR-ABL a3 Positive Control** and **BCR-ABL a2 Negative Control / BCR-ABL a3 Negative Control** in the “Controls” menu. Note: All must have been run, approved and not expired.	**3.** Thaw the **BCR-ABL a2 PCR Mix / BCR-ABL a3 PCR Mix** tubes. Vortex gently. Spin down 5 sec.

4. Prepare the complete reaction mixture

Samples Number (N)	**BCR-ABL a2 PCR Mix and BCR-ABL a3 PCR Mix**	**RT EnzymeMix**
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL
13 ≤ N ≤ 18	(N + 3) x 20 µL	(N + 3) x 0.3 µL
19 ≤ N ≤ 23	(N + 4) x 20 µL	(N + 4) x 0.3 µL
N = 24	580 µL	8.7 µL

5. Vortex gently.
Spin down 5 sec.
Keep the complete reaction mixture in ice. Do not expose to direct light.

Procedure 1 - Complete run: Extract + PCR (e.g., samples)

1. Select “Perform Run” on the touch screen and then click on the run mode “Extract and PCR”.	**2.** Insert the Sample Rack with the samples in the Cooler Unit. Scan the sample barcodes or type the sample ID.	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”
4. Select the “Assay Protocol” of interest: BCR-ABL a2 ELITe_PBL_200_100 and BCR-ABL a3 ELITe_PBL_200_100	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit.	**6.** Load the complete reaction mixture in the Reagent/Elution Rack and insert it in the Cooler Unit.
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Rack” with the “ELITe InGenius SP RNA” extraction cartridges, “ELITe InGenius DNase I tubes” and the required extraction consumables.	**8.** Close the door. Start the run.	**9.** View, approve and store the results.

NOTE

If an Extract Only mode is needed, refer to the instrument user’s manual for procedure.

Procedure 2: PCR Only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen and then click on the run mode “PCR Only”.	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit”.	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “100 µL”.
4. Select the “Assay Protocol” of interest: BCR-ABL a2 ELITe_PBL_200_100 and BCR-ABL a3 ELITe_PBL_200_100 or BCR-ABL a2 ELITe_PC or BCR-ABL a3 ELITe_PC or BCR-ABL a2 ELITe_NC or BCR-ABL a3 ELITe_NC	**5.** Load the Complete reaction mixture in the Reagent/Elution Rack and insert it in the Cooler Unit.	**6.** Load “PCR Rack” with “PCR Cassette”.
7. Close the door. Start the run.	**8.** View, approve and store the results.	

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