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EMPOWERING IVD

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NOTICE of CHANGE dated 20/08/2024

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«Coagulation ELITe MGB® Kit»
Ref. RTSD00ING

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

- *Update for compliance with the Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) requirements and update of the Intended use, accordingly.*

- ***NOTE:** Composition and PERFORMANCE CHARACTERISTICS of the product remain unchanged.*

- ***NOTE:** The product lots reported into the table below are still placed on the market as per IVDD (98/79/EC) until expiration dates, according to Article 110 of IVDR. If you're using these product lots, the related IFU revision, NOT available anymore on the website, can be requested by contacting ELITechGroup staff.*

PRODUCT REF	Lot Number	Expiry date
RTSD00ING	U1123-091	31/08/2025
RTSD00ING	U0524-050	31/07/2025

PLEASE NOTE

	LA REVISIONE DI QUESTA IFU NON È COMPATIBILE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVISION OF THIS IFU IS NOT COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	LA RÉVISION DE CETTE IFU N'EST PAS COMPATIBLE AVEC LA VERSION PRÉCÉDENTE DU KIT
	LA REVISIÓN DE ESTE IFU NO ES COMPATIBLE CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU NÃO ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST NICHT KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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Coagulation ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTSD00ING

UDI 08033891486204

CE 0123

IVD

-20 °C

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INTENDED USE

The product **Coagulation ELITe MGB® Kit** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative nucleic acids Real-Time PCR assay for the allelic discrimination of the following three loci in human genomic DNA samples extracted from clinical specimens:

- coagulation Factor V gene, single nucleotide polymorphism (SNP) G1691A (Leiden),
- coagulation Factor II gene, SNP G20210A,
- 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, SNP C677T.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®**, automated and integrated instruments for extraction, Real-Time PCR and results interpretation, using human specimens of whole blood collected in EDTA.

The product is intended for use as an aid in assessing the risk of deep vein thrombosis in patients suspected of having coagulation disorders and at risk of deep vein thrombosis.

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

ASSAY PRINCIPLES

The assay is a qualitative Real-Time PCR validated on **ELITe InGenius** and **ELITe BeGenius**, automated and integrated systems for extraction, amplification and detection of nucleic acids and results interpretation.

Starting from genomic DNA extracted from the samples being tested, three specific amplification reactions for the three human genes coding for the targets of interest and one specific amplification reaction for the internal control (sample suitability check) are performed in the PCR Cassette:

- Factor V, SNP G1691A (Leiden) region, detected by a specific probe labelled with AP639 fluorophore in the "**FV**" channel of the instrument,
- Factor II, SNP G20210A region, detected by a specific probe labelled with FAM fluorophore in the "**FII**" channel of the instrument,
- MTHFR, SNP C677T region, detected by a specific probe labelled with AP593 fluorophore in "**MTHFR**" channel of the instrument.
- Internal Control, human beta Globin gene region, detected by a probe labeled with AP525 fluorophore in the "**IC**" channel of the instrument.

The probes with ELITe MGB® technology are activated when they hybridize with the specific product of the amplification reaction. **ELITe InGenius** and **ELITe BeGenius** monitors fluorescence increase and calculates the threshold cycles (Ct).

At the end of amplification cycle, the analysis of the melting curve allows to identify the probes melting temperatures and to detect the presence of wildtype and/or mutated alleles.

PRODUCT DESCRIPTION

The **Coagulation ELITe MGB Kit**, product supplies the **52M PCR Mix,** a complete mixture for Real Time amplification, **ready to use** and **aliquoted into eight test tubes**. Each tube contains **280 µL** of solution and is sufficient for **12 tests on ELITe InGenius** and **ELITe BeGenius** if processing at least 2 samples per session.

The **52M PCR Mix** contains the specific primers and probes for:

- the coagulation Factor V gene, SNP G1691A (Leiden) region. The probe is labelled with AP639 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent molecule,
- the coagulation Factor II gene, SNP G20210A region. The probe is labelled with FAM fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent molecule,
- for MTHFR gene, SNP C677T region. The probe is labelled with AP593 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent molecule,
- the IC, region of the human gene encoding for beta Globin. The probe is labeled with AP525 fluorophore, stabilized by the MGB® group and inactivated by the non-fluorescent quencher,

The **52M PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, and hot-start DNA Polymerase.

The product is sufficient for **96 tests** in association with **ELITe InGenius** and **ELITe BeGenius**, with 20 µL used per reaction.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of Hazards
52M PCR Mix	Complete reaction mixture	**8 x 280 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- 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).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples to be analyzed, the amplification positive control and the consumables are **not** included in this product.

For automatic sample analysis with the **ELITe InGenius** (ELITechGroup S.p.A., EG SpA, ref. INT030) or **ELITe BeGenius** (EG SpA, ref. INT040) the following generic products are required:

- extraction cartridges **ELITe InGenius® SP 200** (EG SpA, Ref. INT032SP200),
- extraction consumables **ELITe InGenius® SP 200 Consumable Set** (EG SpA, Ref. INT032CS),
- **ELITe InGenius® Waste Box** (EG SpA, ref. F2102-000),
- **ELITe InGenius® PCR Cassette** (EG SpA, Ref. INT035PCR),
- **300 µL Filter Tips Axygen** (Corning Life Science, ref. TF-350-L-R-S), for **ELITe InGenius**,
- **1000 µL Filter Tips Tecan** (Tecan, ref. 30180118), for **ELITe BeGenius**.

For automatic extraction of nucleic acids, Real-Time PCR and result interpretation of samples with the **ELITe InGenius** instrument, the following specific Assay Protocols are required:

- parameters for the amplification of positive control **52M ELITe_PC**,
- parameters for the amplification of negative control **52M ELITe_NC**,
- parameters for samples to be analyzed **52M ELITe_WB_200_200**.

For automatic extraction of nucleic acids, Real-Time PCR and result interpretation of samples with the **ELITe BeGenius** instrument, the following specific Assay Protocols are required:

- parameters for the amplification of positive control **52M ELITe_Be_PC**,
- parameters for the amplification of negative control **52M ELITe_Be_NC**,
- parameters for samples to be analyzed **52M ELITe_Be_WB_200_200**.

As template of amplification of positive control, the specifc product **Coagulation - ELITe Positive Control** (EG SpA, ref. CTRD00ING), is required. This is a stabilised solution containing plasmid DNAs.

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WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with 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. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in 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 with the product before running the assay.
While running the assay, follow the instructions provided with the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided with the product and those recommended by the manufacturer.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

Lab 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 pipettes used to handle extraction products must be exclusively used for this purpose.

The PCR Cassettes must be handled in such a way to reduce as much as possible amplification product diffusion into the environment in order to avoid sample and reagent contamination.

Warnings and precautions specific for the components:

The **52M PCR Mix** must be stored at -20 °C or below and protected from light.
The **52M PCR Mix** must be used within one month from the first opening.
The **52M PCR Mix** can be frozen and thawed for no more than **seven times**: further freezing / thawing cycles may cause a loss of product performance.

The **52M PCR Mix** can be kept on board in the inventory area cool block of ELITe InGenius or in the Cool Unit of ELITe BeGenius up to **seven separate sessions of three hours each** (Extract + PCR mode), or for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total). Gently mix and centrifuge the contents for 5 seconds before starting the next session.

ELITe InGenius

SAMPLES AND CONTROLS

Samples

This product must be used with whole blood samples collected in EDTA.

The whole blood samples for DNA extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2 / +8° C and stored at +2 / +8° C for a maximum of three days, otherwise they must be frozen and stored at -20 or -70 °C or below for a maximum of thirty days. Even if longer storage periods at -70 °C or below, as it is extensively reported by scientific literature, are possible, their application should be evaluated internally by the end-user of this product.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when the DNA extraction from whole blood samples is carried out with the ELITe InGenius and with ELITe InGenius Software version 1.2 (or later equivalent versions) use the Assay protocol **52M ELITe_WB_200_200**. This protocol processes 200 µL of sample and elutes the nucleic acids in 200 µL. When a primary tube (13 x 7.5 mm) is used, sample volume must be at least of 2.2 mL.

Other samples

There are no data available concerning product performance with DNA extracted from the following clinical samples: saliva.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in paragraph “Interfering substances” of chapter “Performance characteristics”.

Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the amplification controls for the amplification reagent lot that will be used in testing:

- as a Positive Control amplification, use the **Coagulation - ELITe Positive Control** (product not provided with this kit) in association with protocol **52M ELITe_PC**
- as a Negative Control amplification, use molecular grade water (not provided with this kit) in association with protocol **52M ELITe_NC**,

Note: The ELITe InGenius requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.
The amplification control results, approved and stored in the database, will expire **after 15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot.

Furthermore, the amplification controls must be re-run when:

- a new lot of amplification reagents is started,
- the results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance service is performed on the ELITe InGenius instrument.

Quality controls

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

Coagulation ELITe MGB® Kit
reagent for Real Time del DNA amplification

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PROCEDURE

Using the **Coagulation ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and approval of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the ELITe InGenius and select the login mode “**CLOSED**”,

- verify that the amplification controls (52M Positive Control, 52M Negative Control) were run in association with the amplification reagent lot to be used and that they are approved and valid (Status). If there are not amplification controls approved or valid, run them as described in the following paragraphs,

- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA. These IVD protocols were specifically validated with ELITe MGB kits, the **ELITe InGenius** instrument and the cited matrix.

The Assay protocol available for sample testing with the **Coagulation ELITe MGB Kit** product is described in the table below.

Assay protocol for Coagulation ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
52M ELITe_WB_200_200	Whole Blood	Wild-type/ heterozygous/ homozygous	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Setup of the session

The **Coagulation ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

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

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

Note: The **ELITe InGenius** can be linked to the “Location Information Server” (LIS) which enables loading the session information. Refer to the **ELITe InGenius** instrument user’s manual for more details.

The main steps for the setup of the three types of runs are described here below.

A Integrated run

To setup an integrated run, carry out the following steps as per the Graphic User Interface (GUI):

1. Thaw samples at room temperature (+21 ±5 °C) and handle according to laboratory guidelines and to the “Samples and Controls” section.
2. Thaw at room temperature (+21 ±5 °C) for 30 minutes **52M PCR Mix** tubes for the session. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently, spin down the content for 5 seconds.

Note: Protect the **52M PCR Mix** from light while thawing because this reagent is photosensitive.

3. Select “Perform Run” from the “Home” screen.
4. Ensure that the “Extraction Input Volume” is 200 µL and the Extracted Elute Volume is 200 µL.
5. For each Track of interest fill in the “SampleID” (SID) by typing or by scanning the sample barcode.
6. Select the Assay protocol to be used in the “Assay” column (i.e. 52M ELITe_WB_200_200).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select the sample loading position in the “Sample Position” column:
   - if a primary tube is used, select “Primary Tube”,
   - if a secondary tube is used, select “Extraction Tube”.

   Click “Next” to continue the setup.
9. Load **52M PCR Mix** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **52M PCR Mix**. Click “Next” button to continue the setup.
10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” button to continue the setup.
11. Load the “**PCR Cassettes**”, the “**ELITe InGenius SP 200**” extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results and to print and save the report.

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be stored in the refrigerated block for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total). Gently mix and centrifuge the contents for 5 seconds before starting the next session.

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B Amplification run

To set up the amplification run starting from extracted DNA, follow the steps below while referring to the GUI:

1. Thaw at room temperature (+21 ±5 °C) for 30 minutes **52M PCR Mix** tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Protect the **52M PCR Mix** from light while thawing because this reagent is photosensitive.

2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 200 µL.
4. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
5. Select the Assay protocol to be used in the “Assay” column (i.e. 52M ELITe_WB_200_200).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load **52M PCR Mix** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **52M PCR Mix**. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “**PCR Cassette**” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results and to print and save the report.

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be stored in the refrigerated block for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total) Gently mix and centrifuge the contents for 5 seconds before starting the next session.

C. Amplification run for Positive Control and Negative Control

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

1. Thaw at room temperature (+21 ±5 °C) for 30 minutes **52M PCR Mix** tubes for the session. Each tube is sufficient for preparing 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

   Note: Thaw **52M PCR Mix** in the dark as the reagent is photosensitive.
2. Thaw the **52M Positive Control** tube for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water to an "Elution tube", provided with the "ELITe InGenius® SP 200 Consumable Set".
4. Select "Perform Run" from the "Home" screen.
5. In the Track of interest, select the Assay protocol to be used in the "Assay" column.
6. For the positive control, select the Assay Protocol 52M ELITe_PC in the "Assay" column and fill in the lot number and expiry date of 52M Positive Control.
7. For the negative control, select the Assay Protocol 52M ELITe_NC in the "Assay" column and fill in the lot number and expiry date of the molecular biology grade water.
8. Click "Next" to continue the setup.
9. Load **52M PCR Mix** on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of **52M PCR Mix**. Click "Next" to continue the setup.
10. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load the "**PCR Cassettes**", the "**52M Positive Control**" tube and the negative control tube following the GUI instruction. Click "Next" to continue the setup.
12. Close the instrument door.
13. Press "Start" to start the run.

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

Note: At the end of the run the remaining Positive Control must be removed from the instrument, capped, identified and stored at -20 °C or below. Avoid spilling the Extracted Sample. The remaining Negative Control must be disposed.

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be stored in the refrigerated block or for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total). Gently mix and centrifuge the contents for 5 seconds before starting the next session.

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Review and approval of results

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

Note: The ELITe InGenius can be connected to the "Location Information Server" (LIS) enables sending the session results to the laboratory data center. Refer to the **ELITe InGenius** instrument user's manual for more details.

The ELITe InGenius generates the results with the product **Coagulation ELITe MGB Kit** through the following procedure:

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

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of the three genes (**FV**, **FII**, **MTHFR** Channels) and for the IC (**IC** Channel) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the **ELITe InGenius** software with the parameters included in the Assay protocols "52M ELITe_PC" and "52M ELITe_NC".

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

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

Before analyzing any sample, it is mandatory to verify that Positive Control and Negative Control results are approved and valid for the PCR reagent lot. The Status of Positive Control and Negative Control results for each lot of PCR reagent is shown in the "Controls" module. If the results of Positive Control and/or Negative Control are missing or expired, run the control(s) as described above.

The **ELITe InGenius software** processes the Positive Control and Negative Control results and generates Control Charts. Four approved Positive Control and Negative Control results are used to set up the initial Control Chart. For subsequent controls, the results are analyzed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. 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.

B. Validation of Sample results

The fluorescence signals emitted by the probes of the three genes (**FV**, **FII**, **MTHFR** Channels) and for the IC (**IC** Channel) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocol.

The **ELITe InGenius software** interprets the PCR results for the three genes (FV, FII, MTHFR Channels) and the Internal Control probe (Channel **IC**) with the 52M ELITe_WB_200_200 Assay Protocol parameters.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the two conditions reported in the table below are true.

1) Positive Control	**Status**
52M Positive Control	APPROVED
2) Negative Control	**Status**
52M 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 pathogen DNAs are either detected or not detected.

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

Result of sample run	Interpretation
FV: Wildtype for Factor V	Sample has a **wildtype** genotype for Factor V SNP G1691A locus.
FV: Heterozygous for Factor V Leiden	Sample has a **mutated (Leiden) heterozygous** genotype for Factor V SNP G1691A locus.
FV: Homozygous for Factor V Leiden	Sample has a **mutated (Leiden) homozygous** genotype for Factor V SNP G1691A locus.
FII: Wildtype for Factor II	Sample has a **wildtype** genotype for Factor II SNP G20210A locus.
FII: Heterozygous for Factor II 20210A	Sample has a **mutated (20210A) heterozygous** genotype for Factor II SNP G20210A locus.
FII: Homozygous for Factor II 20210A	Sample has a **mutated (20210A) homozygous** genotype for Factor II SNP G20210A locus.
MTHFR: Wildtype for MTHFR	Sample has a **wildtype** genotype for MTHFR SNP C677T locus.
MTHFR: Heterozygous for MTHFR 677T	Sample has a **mutated (677T) heterozygous** genotype for MTHFR SNP C677T locus.
MTHFR: Homozygous for MTHFR 677T	Sample has a **mutated (677T) homozygous** genotype for MTHFR SNP C677T locus.
IC: Valid Sample	Sample has a Ct value of the IC of less than 26.5 and is valid
Inconclusive - Retest Sample	**Inconclusive** assay result due to a sample problem.
Invalid - Retest Sample	**Not valid** assay result due to a problem of incorrect extraction or inhibitor carry-over.

Samples reported as “Inconclusive – Retest Sample” are not suitable for result interpretation, which it is not been possible to detect the melting temperature (Tm) of wildtype and mutated alleles. “In this case, the dissociation curve analysis was not efficiently carried out due to problems with sample (inhibitors carry-over in the eluate or presence of other interfering SNPs), which may cause incorrect results.

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Samples not suitable for result interpretation are reported as “Invalid - Retest Sample” by the **ELITe InGenius** software. In this case, the human genomic DNA of the sample was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction, inhibitors carry-over in the eluate or DNA quantity in the sample not sufficient), which may cause incorrect results.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

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

Note: when approving the assay results, always verify the instrument outcome by checking the melting curve plots in the work session report. The melting temperatures (Tm) of wildtype and/or mutated alleles of each gene in analysis have to correspond to the peaks showed in the melting curve plots.

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

C. Sample result reporting

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

The “Sample Report” shows the details of a work session sorted by selected sample (SID).

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

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

ELITe BeGenius

SAMPLES AND CONTROLS

Samples

This product must be used with whole blood samples collected in EDTA.

The whole blood samples for DNA extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2 / +8° C and stored at +2 / +8° C for a maximum of three days, otherwise they must be frozen and stored at -20 or -70 °C or below for a maximum of thirty days. Even if longer storage periods at -70 °C or below, as it is extensively reported by scientific literature, are possible, their application should be evaluated internally by the end-user of this product.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when the DNA extraction from whole blood samples is carried out on the ELITe BeGenius and with ELITe BeGenius Software version 2.1.0 (or later equivalent versions) use the Assay protocol **52M ELITe_Be_WB_200_200**. This protocol processes 200 µL of sample and elutes the nucleic acids in 200 µL. When a primary tube (13 x 75 mm, 13 x 100 mm or 16 x 100 mm) is used, sample volume must be at least of 1.5 mL.

Other samples

There are no data available concerning product performance with DNA extracted from the following clinical samples: saliva.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in paragraph “Interfering substances” of chapter “Performance characteristics”.

Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the amplification controls for the amplification reagent lot that will be used in testing:

- as a Positive Control amplification, use the **Coagulation - ELITe Positive Control** (product not provided with this kit) in association with protocol **52M ELITe_Be_PC**,
- as a Negative Control amplification, use molecular grade water (not provided with this kit) in association with protocol **52M ELITe_Be_NC**.

Note: The ELITe BeGenius instrument requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.
The amplification control results, approved and stored in the database, will expire **after 15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot.
Furthermore, the amplification controls must be re-run when:

- a new lot of amplification reagents is started,
- the results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance service is performed on the ELITe BeGenius instrument.

Quality controls

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

PROCEDURE

Using the **Coagulation ELITe MGB Kit** with **ELITe BeGenius** consists of three steps:

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

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the ELITe BeGenius and select the login mode "**CLOSED**",
- verify that the amplification controls (52M Positive Control, 52M Negative Control) are approved and valid (Status) for the **52 M PCR Mix** lot to be used. If no valid amplification Controls are available for the **52 M PCR Mix** lot, run the amplification Controls as described below.
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A. These IVD protocols were specifically validated with ELITe MGB Kits, the **ELITe BeGenius** instrument and the cited matrix.
- The Assay Protocol available for sample testing with the product **Coagulation ELITe MGB Kit** is described in the table below:

Assay Protocol for Coagulation ELITe MGB Kit			
Name	**Matrix**	**Report**	**Characteristics**
52M ELITe_Be_WB_200_200	Whole Blood	Wild-type/ heterozygous/ homozygous	Extraction Input Volume: 200 µL Extraction Elute Volume: 200 µL Internal Control: NO Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

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Session Setup

The **Coagulation ELITe MGB Kit** in association with the **ELITe BeGenius**, can be used in order to perform:

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

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

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

The main steps for the setup of the three types of runs are described here below.

A Integrated run

To set up an integrated run with sample extraction and amplification, follow the steps below while referring to the GUI:

1. Thaw samples at room temperature (+21 ±5 °C) and handle according to laboratory guidelines and to the "Samples and Controls" section.
2. Thaw the needed **52M PCR Mix** tubes at room temperature (+21 ±5 °C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the content for 5 seconds.

Note: Protect the **52M PCR Mix** from light while thawing because this reagent is photosensitive.

3. Select "Perform Run" from the "Home screen".
4. Remove the racks from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "Extract + PCR".
6. Load the specimens into Racks 5 and 4 (always starting from Rack 5), using adaptors for appropriate fit if necessary.
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue.

Note: If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the sample ID.

8. Ensure that the "Extraction Input Volume" is 200 µL and the "Extraction Elution Volume" is 200 µL.
9. Select the assay protocol to be used in the "Assay" column (i.e. 52M ELITe_Be_WB_200_200). Click "Next" to continue.
10. If Rack 4 is used, repeat step 7 to 9.
11. Load the Elution tubes into the Racks 3 and 2 (start always from Rack 3).

Note: Elution tubes can be labelled to improve traceability.

12. Insert the Rack into the "Cooler Unit". Click "Next" to continue.
13. If Rack 2 used, repeat step 12.
14. Load **52M-PCR Mix** into the Rack 1.
15. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue.
16. Verify the tips in the Tip Racks in the "Inventory Area" and replace Tip Rack(s) if necessary. Click "Next" to continue.
17. Load the Basket with **PCR Cassette** in the "Inventory Area" by following the GUI instruction. Click "Next" to continue.
18. Load the Basket with the **ELITe InGenius SP 200** extraction cartridges and the required extraction consumables by following the GUI instruction. Click "Next" to continue.

19. Close the instrument door.
20. Press “Start” to start the run.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, and store the results and to print and save the report.

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

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

B Amplification run

To set up the amplification run starting from extracted DNA, follow the steps below while referring to the GUI:

1. If needed, thaw eluted samples at room temperature (+21 ±5 °C). Mix gently then spin down the contents for 5 seconds.
1. Thaw the needed **52M PCR Mix** tubes at room temperature (+21 ±5 °C) for 30 minutes. Each tube is sufficient for 24 reactions in optimized conditions (2 or more tests per session). Mix gently, and spin down the contents for 5 seconds.

Note: Protect the **52M PCR Mix** from light while thawing because this reagent is photosensitive.

2. Select “Perform Run” on the “Home screen”.
3. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
4. Select the run mode: “PCR Only”.
5. Load the samples into the Racks 3 and 2 (start always from Rack 3).
6. Insert the rack into the “Cooler Unit”. Click “Next” to continue.
7. Ensure that the “Extraction Input Volume” is 200 µL and the “Extraction Elution Volume” is 200 µL, even if extraction is not being performed.
8. Select the assay protocol to be used in the “Assay” column (e.g. 52M ELITe_Be_WB_200_200). Click “Next” to continue.
9. If Rack 2 is used, repeat steps 7 to 9.
10. Load **52M PCR Mix** into Rack 1.
11. Insert the rack into the “Cooler Unit”. Click “Next” to continue.
12. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
13. Load the basket with **PCR Cassette** in the “Inventory Area” by following the GUI instruction. Click “Next” to continue.
14. Close the instrument door.
15. Press “Start” to start the run.

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

Note: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at ~-20 °C or below. Avoid the spilling of the Extracted Sample.

Note: At the end of the run the “**PCR Cassette**” with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

C. Amplification run for Positive Control and Negative Control

To setup the amplification run for Positive Control and Negative Control, follow the steps below while referring to the GUI:

1. Thaw the needed **52M PCR Mix** tubes at room temperature (+21 ±5 °C) for 30 minutes. Each tube is sufficient for 12 reactions in optimized conditions (2 or more tests per session). Mix gently, then spin down the content for 5 seconds.

Note: Protect the **52M PCR Mix** from light while thawing because this reagent is photosensitive.

2. Thaw **52M Positive Control** tubes at room temperature (+21 ±5 °C) for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently, then spin down the content for 5 seconds.
3. Transfer ≥50 µL of the molecular biology grade water (as Negative Control) in an Elution tube, provided with the **ELITe InGenius SP Consumable Set**.
4. Select “Perform Run” from the “Home screen”.
5. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
6. Select the run mode: “PCR Only”.
7. Load the Positive Control and Negative Control tubes into Racks 3.
8. Insert the rack into the “Cooler Unit”. Click “Next” to continue.
9. Select the assay protocol to be used in the “Assay” column (52M ELITe_Be_PC and 52M ELITe_Be_NC). Click “Next” button to continue.
10. Load the **52M PCR Mix** into Rack 2.
11. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
12. Verify the tips in the Tip Racks in the “Inventory Area” and replace Tip Rack(s) if necessary. Click “Next” to continue.
13. Load the Basket with **PCR Cassette** in the “Inventory Area” by following the GUI instruction. Click “Next” to continue.
14. Close the instrument door.
15. Press “Start” to start the run.

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

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.

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

Note: The PCR Mix can be used for 7 independent work sessions of 3 hours each or can be kept on board in the refrigerated block for 2 sessions of 3 hours each (Extract + PCR mode) and for the time needed to start a third session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Control results and the information regarding the run are shown. From this screen is possible to approve the result, print or save the reports ("Sample Report" or "Track Report").

The ELITe BeGenius generates the results using the **Coagulation ELITe MGB Kit** through the following procedure:

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

Note: please, refer to the same **ELITe InGenius** chapters for the details.

PERFORMANCE CHARACTERISTICS
ELITe InGenius and ELITe BeGenius

Efficiency of detection (inclusivity)

The efficiency of detection of Factor V, Factor II and MTHFR genes was evaluated by comparison of sequences with nucleotide database.

The analysis of the regions chosen for the hybridisation of the primers in the alignment of the sequences available in the database showed their conservation and absence of significant mutations.

The fluorescent probes allow to detect the alleles of the Factor V, Factor II and MTHFR genes reported in the following table. The limits of the related Tm intervals for ELITe InGenius were calculated with data obtained in the verification and validation studies and the Tm ranges in use are shown in the following table. The Tm intervals for ELITe BeGenius were calculated analysing data obtained in the verification study related to extension of use of the product on ELITe BeGenius.

Gene	Allele detected	Tm range	
		ELITe InGenius	ELITe BeGenius
Factor V	Allele 1691G (wild type)	53.0 °C – 57.0 °C	52.5 °C – 56.5 °C
	Allele 1691A (mutated, Leiden)	61.0 °C – 65.0 °C	59.5 °C – 63.5 °C
Factor II	Allele 20210G (wild type)	56.0 °C – 61.0 °C	55.0 °C – 60.0 °C
	Allele 20210A (mutated)	64.0 °C – 69.0 °C	63.0 °C – 68.0 °C
MTHFR	Allele MTHFR 677C (wild type)	55.0 °C – 59.0 °C	54.0 °C – 58.0 °C
	Allele MTHFR 677T (mutated)	64.0 °C – 68.0 °C	63.0 °C – 67.0 °C

Analytical Sensitivity:

The Analytical Sensitivity of the Coagulation ELITe MGB Kit was defined on **ELITe InGenius** (PCR Only mode) and it was verified on **ELITe BeGenius** (PCR Only mode).

The analytical sensitivity of this assay, allows to identify the presence of about 20,000 molecules of target DNA (corresponding to the genomes of ~ 10,000 cells or ~ 70 ng of human genomic DNA) in 20 µL of extracted DNA in reaction.

The analytical sensitivity was verified using panels of human genomic DNA certified for Factor V and Factor II (WHO Reference Reagent Factor V Leiden, Human gDNA, 1st International Genetic International Panel, NIBSC, UK, code 04/224, and WHO Reference Reagent Prothrombin Mutation G20210A, Human gDNA, 1st International Genetic International Panel, NIBSC, UK, code 05/130)).

The three samples (wild type, heterozygous and homozygous) of each panel were tested in 4 replicates at about 70 ng per reaction to perform the amplification and detection procedure with the product and the **ELITe InGenius** and **ELITe BeGenius**. All replicates resulted valid and correctly determined.

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Analytical Specificity

The Analytical Specificity of this assay, as the ability of not identifying as Factor II mutated for SNP G20210A the gene mutated for SNP C20209T, was verified using a plasmid DNA containing the Factor II amplicon with wildtype nucleotide (G) in position 20210 and the mutant nucleotide (T) in position 20209.

A simulated sample containing about 80,000 copies of plasmid DNA was used in 24 replicates to perform the amplification and detection procedure with the product and the ELITe InGenius. All replicates were correctly determined: no sample mutated for Factor II SNP C20209T was called as heterozygous or homozygous mutated for Factor II SNP G20210A.

Moreover, the Analytical Specificity was verified for the following uncommon SNPs described in scientific literature. These are rare SNPs that fall in proximity of the SNPs of interest:

Gene	rare SNP analysed
Factor V	G1689A, C1690T, A1692C, A1696G
Factor II	A20207C, A20218G, T20219A, C20221T
MTHFR	C678A, G679A, C684G

The Analytical Specificity has been verified using plasmid DNAs containing the amplified region of Factor V, Factor II and MTHFR genes with wildtype nucleotide in the SNPs of interest and the mutant nucleotide in the rare SNPs in analysis.

Simulated samples containing about 80,000 copies of plasmid DNA were used in 6 replicates to perform the amplification and detection procedure with the product and the ELITe InGenius. The replicates resulted "Inconclusive" or correctly determined. No sample mutated for the rare SNPs in analysis was called as heterozygous or homozygous mutated for the SNPs of interest.

Rare SNPs falling within the probe hybridization region may interfere with the detection of the SNP of interest and could lead to "false homozygous mutant" result when occurring together with the mutation of the SNP of interest on the other allele.

Potential interfering organisms: cross-reactivity

The potential Cross-reactivity with other organisms that might be found in clinical samples of Whole Blood was evaluated by *in silico* analysis of the sequences available in nucleotide databases.

The analysis of the amplified region of the product sequences showed absence of significant homologies. So, no cross-reactivity by potential interfering organisms is expected.

Potential interfering organisms: inhibition

The Absence of inhibition by other organisms that might be found in clinical samples of Whole Blood was evaluated by *in silico* analysis of the sequences available in nucleotide databases.

The analysis of the amplified region of the product sequences showed absence of significant homologies. So, no inhibition by potential interfering organisms is expected.

Interfering substances

The effect of potentially interfering substances was evaluated by analyzing a whole blood sample collected in EDTA, heterozygous for the three genes of interest, spiked with the following potential interfering substances: Bilirubin 300 µg / mL, Triglycerides 4 mg / mL, Heparin 8.3 µg / mL, 50 mM EDTA, Ibuprofen 100 µg / mL, Ganciclovir 10 µg / mL, Ampicillin 18 µg / mL and Cyclosporine 0.3 µg / mL).

The substance spiked samples and a reference sample (not-spiked) were used to perform the entire analysis, extraction and amplification procedure in 3 replicates, with the product and the ELITe InGenius. All replicates were valid and correctly genotyped.

The results are summarized in the following table:

Sample	Genotype FV het / FII het / MTHFR het	IC Ct (Cut-off = 26.5) replicates			Outcome
		1	2	3	
Control	3 / 3	22.72	22.42	22.25	No interference
Cyclosporine A	3 / 3	22.55	22.17	22.31	No interference
Ganciclovir	3 / 3	22.47	22.87	22.35	No interference
Heparin	3 / 3	22.31	22.67	22.31	No interference
EDTA	3 / 3	23.27	23.46	23.41	No interference
Ibuprofen	3 / 3	24.98	23.77	23.59	No interference
Triglycerides	3 / 3	23.42	24.49	24.51	No interference
Ampicillin	3 / 3	22.59	22.69	22.26	No interference
Bilirubin	3 / 3	22.73	22.61	22.35	No interference

Repeatability

The Repeatability of results obtained by the Coagulation ELITe MGB Kit product in association with the **ELITe InGenius** and **ELITe BeGenius** was tested by analysing a whole blood panel collected in EDTA consisting of 3 samples:

- genotype FII SNP G1691A heterozygous (Leiden) (normal FV and normal MTHFR),
- genotype FV SNP G20210A heterozygous (normal FII and heterozygous MTHFR),
- genotype MTHFR SNP C677T heterozygous (normal FII and normal FV).

The Intra – Session Repeatability was obtained through the analysis of 3 samples of the panel, each heterozygous for the SNP of interest of one gene, in 8 replicates in one run in one run per day, with the same lot of product, with the same instrument, on the same day. Samples were processed in randomized positions in "Extract + PCR" mode.

The Inter – Session Repeatability was obtained through the analysis of 3 samples of the panel, each heterozygous for the SNP of interest of one gene, in 8 replicates, in one run per day, with the same lot of product, with the same instrument, on two different days. Samples were processed in randomized positions in "Extract + PCR" mode.

All replicates were valid and correctly determined. The variability of the results obtained was calculated as %CV of IC Ct values and of Tm of the three gene of interest.

The results are summarized in the following tables.

Intra - Session Repeatability on ELITe InGenius				
Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G1691A	IC	8	22.07	0.41
Heterozygous FV SNP G20210A		8	22.91	0.76
Heterozygous MTHFR SNP C677T		8	22.02	1.22

Intra - Session Repeatability on ELITe InGenius				
Panel sample	Allele	N	Mean Tm	%CV Tm
Heterozygous FII SNP G1691A	Factor II wild type	8	58.19	0.207
	Factor II mutated	8	65.66	0.051
Heterozygous FV SNP G20210A	Factor V wild type	8	55.18	0.048
	Factor V mutated	8	62.31	0.120
Heterozygous MTHFR SNP C677T	MTHFR wild type	8	56.73	0.183
	MTHFR mutated	8	65.90	0.169

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Inter -Session Repeatability on ELITe InGenius				
Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G1691A	IC	16	22.12	1.12
Heterozygous FV SNP G20210A		16	22.91	0.75
Heterozygous MTHFR SNP C677T		16	22.10	1.36

Inter - Session Repeatability on ELITe InGenius				
Panel sample	Allele	N	Mean Tm	%CV Tm
Heterozygous FII SNP G1691A	Factor II wild type	16	58.18	0.182
	Factor II mutated	16	65.78	0.258
Heterozygous FV SNP G20210A	Factor V wild type	16	55.18	0.064
	Factor V mutated	16	62.31	0.116
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.73	0.211
	MTHFR mutated	16	65.96	0.257

The Repeatability on **ELITe InGenius** of the product Coagulation ELITe MGB Kit showed Ct values of IC with %CV below 5% and a Tm value of the three genes of interest lower than 5%.

Intra - Session Repeatability on ELITe BeGenius				
Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G1691A	IC	8	22.35	1.90
Heterozygous FV SNP G20210A		8	22.95	2.20
Heterozygous MTHFR SNP C677T		8	22.40	1.28

Intra - Session Repeatability on ELITe BeGenius				
Panel sample	Allele	N	Mean Tm	%CV Tm
Heterozygous FII SNP G1691A	Factor II wild type	8	57.64	0.392
	Factor II mutated	8	65.21	0.150
Heterozygous FV SNP G20210A	Factor V wild type	8	54.84	0.120
	Factor V mutated	8	61.56	0.242
Heterozygous MTHFR SNP C677T	MTHFR wild type	8	56.29	0.174
	MTHFR mutated	8	65.30	0.116

Inter - Session Repeatability on ELITe BeGenius				
Panel sample	Target	N	Mean Ct	%CV Ct
Heterozygous FII SNP G1691A	IC	16	22.26	1.58
Heterozygous FV SNP G20210A		16	23.10	2.02
Heterozygous MTHFR SNP C677T		16	22.45	1.75

Inter - Session Repeatability on ELITe BeGenius				
Panel sample	Allele	N	Mean Tm	%CV Tm
Heterozygous FII SNP G1691A	Factor II wild type	16	57.71	0.494
	Factor II mutated	16	65.24	0.119
Heterozygous FV SNP G20210A	Factor V wild type	16	54.82	0.131
	Factor V mutated	16	61.60	0.218
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.29	0.273
	MTHFR mutated	16	65.28	0.099

The Repeatability on **ELITe BeGenius** of the product Coagulation ELITe MGB Kit showed Ct values of IC with %CV below 5% and a Tm value of the three genes of interest lower than 5%.

Reproducibility

The Reproducibility of results obtained by the Coagulation ELITe MGB Kit product in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a whole blood panel collected in EDTA consisting of 3 samples:

- genotype FII SNP G1691A heterozygous (Leiden) (normal FV and normal MTHFR),
- genotype FV SNP G20210A heterozygous (normal FII and heterozygous MTHFR),
- genotype MTHFR SNP C677T heterozygous (normal FII and normal FV).

The Inter – Batch Reproducibility was obtained through the analysis of 3 samples of the panel, each heterozygous for the SNP of interest of one gene, in 8 replicates per day with 2 different lots and the same instrument in 2 days, samples were processed in randomized positions in "Extract + PCR" mode.

The Inter – Instrument Reproducibility was obtained through the analysis of 3 samples of the panel, each heterozygous for the SNP of interest of one gene, in 8 replicates per day using 2 different instruments, with 2 different operators, in 2 days samples were processed in randomized positions in "Extract + PCR" mode.

All replicates were valid and correctly determined. The variability of the results obtained was calculated as %CV of IC Ct values and of Tm of the three gene of interest.

The results are summarized in the following tables.

Inter – Batch Reproducibility on ELITe InGenius				
Panel sample	**Target**	**N**	**Mean Ct**	**CV% Ct**
Heterozygous FII SNP G1691A	IC	16	22.28	1.28
Heterozygous FV SNP G20210A		16	22.46	2.58
Heterozygous MTHFR SNP C677T		16	23.14	1.47

Inter – Batch Reproducibility on ELITe InGenius				
Panel sample	**Allele**	**N**	**Mean Tm**	**CV% Tm**
Heterozygous FII SNP G1691A	Factor II wild type	16	58.1	0.23
	Factor II mutated	16	65.7	0.12
Heterozygous FV SNP G20210A	Factor V wild type	16	55.1	0.10
	Factor V mutated	16	62.3	0.13
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.6	0.32
	MTHFR mutated	16	65.8	0.22

Inter – Instrument Reproducibility on ELITe InGenius				
Panel sample	**Target**	**N**	**Mean Ct**	**CV% Ct**
Heterozygous FII SNP G1691A	IC	16	22.38	1.53
Heterozygous FV SNP G20210A		16	22.45	2.34
Heterozygous MTHFR SNP C677T		16	23.13	1.27

Inter – Instrument Reproducibility on ELITe InGenius				
Panel sample	**Allele**	**N**	**Mean Tm**	**CV% Tm**
Heterozygous FII SNP G1691A	Factor II wild type	16	58.1	0.27
	Factor II mutated	16	65.6	0.12
Heterozygous FV SNP G20210A	Factor V wild type	16	55.1	0.21
	Factor V mutated	16	62.2	0.16
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.6	0.35
	MTHFR mutated	16	65.7	0.28

The Reproducibility on **ELITe InGenius** of the product Coagulation ELITe MGB Kit showed Ct values of IC with %CV below 5% and a Tm value of the three genes of interest lower than 5%.

Coagulation ELITe MGB® Kit
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Inter – Batch Reproducibility on ELITe BeGenius				
Panel sample	**Target**	**N**	**Mean Ct**	**CV% Ct**
Heterozygous FII SNP G1691A	IC	16	22.42	1.72
Heterozygous FV SNP G20210A		16	22.60	1.99
Heterozygous MTHFR SNP C677T		16	22.80	2.20

Inter – Batch Reproducibility on ELITe BeGenius				
Panel sample	**Allele**	**N**	**Mean Tm**	**CV% Tm**
Heterozygous FII SNP G1691A	Factor II wild type	16	57.6	0.29
	Factor II mutated	16	65.2	0.11
Heterozygous FV SNP G20210A	Factor V wild type	16	54.8	0.11
	Factor V mutated	16	61.5	0.24
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.2	0.27
	MTHFR mutated	16	65.2	0.28

Inter – Instrument Reproducibility on ELITe BeGenius				
Panel sample	**Target**	**N**	**Mean Ct**	**CV% Ct**
Heterozygous FII SNP G1691A	IC	16	22.51	1.60
Heterozygous FV SNP G20210A		16	22.63	1.68
Heterozygous MTHFR SNP C677T		16	23.15	1.83

Inter – Instrument Reproducibility on ELITe BeGenius				
Panel sample	**Allele**	**N**	**Mean Tm**	**CV% Tm**
Heterozygous FII SNP G1691A	Factor II wild type	16	57.7	0.45
	Factor II mutated	16	65.2	0.13
Heterozygous FV SNP G20210A	Factor V wild type	16	54.8	0.16
	Factor V mutated	16	61.6	0.23
Heterozygous MTHFR SNP C677T	MTHFR wild type	16	56.2	0.30
	MTHFR mutated	16	65.3	0.17

The Reproducibility on **ELITe BeGenius** of the product Coagulation ELITe MGB Kit showed an IC amplification Ct value %CV lower than 5% and a Tm value %CV of the three genes of interest lower than 5%.

Robustness: Cross-contamination test

The absence of cross-contamination was verified by analysing whole blood samples alternated to molecular biology grade water.

In this test 6 whole blood samples were alternated to 6 samples of water and they were used to perform the entire analysis, extraction and amplification procedure, with the Coagulation ELITe MGB Kit product and ELITe InGenius in five different sessions.

The results are summarized in the following table.

Samples	**N**	**Genotype**			**IC**
		FII het	**MTHFR wt**	**FV wt**	**Ct < 26.5**
Whole blood collected in EDTA	30	30/30	30/30	30/30	30/30
Molecular biology grade water	30	NA	NA	NA	0/30

The assay called as a "Invalid" any sample of water tested showing the absence of cross-contamination.

Diagnostic Agreement: confirmation of certified sample genotype

The diagnostic agreement of this assay, as the ability to correctly identify the genotype of certified sample, was tested on clinical whole blood samples of subjects with known genotype and, for mutated homozygous genotypes of Factor II SNP G20210A and MTHFR SNP C677T loci, on simulated samples.

The diagnostic agreement was verified using 219 whole blood samples collected in EDTA from different subjects whose genotype was determined by validated reference real time PCR assays and 92 simulated samples with mutated homozygous genotype, prepared with plasmid DNA mixtures in plasma matrix.

The samples were tested by extraction and amplification procedure using the product and the ELITe InGenius.

The results are summarized in the following tables.

Sample genotype: Factor V G1691A	N	WT	Het.	Hom.	Diagnostic Agreement	Total Diagnostic Agreement
Wild type	108	108	0	0	100%	100%
Heterozygous	57	0	57	0	100%	
Homozygous mutated	53	0	0	53	100%	

In the allelic discrimination of the Factor V SNP G1691A locus, the assay returned concordant results for all samples tested. In this test the total diagnostic agreement for the Factor V SNP G1691A locus was equal to 100%.

Sample genotype: Factor II G20210A	N	WT	Het.	Hom.	Diagnostic Agreement	Total Diagnostic Agreement
Wild type	160	160	0	0	100%	100%
Heterozygous	59	0	59	0	100%	
Homozygous mutated (simulated)	56	0	0	56	100%	

In allelic discrimination of the Factor II SNP G20210A locus, the assay returned concordant results for all samples tested. In this test the total diagnostic agreement for the Factor II G20210A locus was equal to 100%.

Sample genotype: MTHFR C677T	N	WT	Het.	Hom.	Diagnostic Agreement	Total Diagnostic Agreement
Wild type	63	63	0	0	100%	100%
Heterozygous	136	0	136	0	100%	
Homozygous mutated	19	0	0	19	100%	
Homozygous mutated (simulated)	36	0	0	36		

In allelic discrimination of the MTHFR SNP C677T locus, the assay returned concordant results for all samples tested. In this test the total diagnostic agreement for the MTHFR C677T locus was equal to 100%.

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 performances of the assay obtained in association with ELITe InGenius is also applicable to **ELITe BeGenius**.

The Internal Control Ct (IC Ct) cut-off value is set at 26.5 for **ELITe InGenius and ELITe BeGenius**.

NOTE.: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in Product Technical File of "Coagulation ELITe MGB Kit", FTP RTSD00ING.

REFERENCES

Voorberg, J. et al. (1994) *The Lancet* 343: 1535 - 1536.
Baker, R. et al. (1994) *The Lancet* 344: 1162.
Poort, S. R. et al. (1996) Blood 88: 3698 - 3703.
Kluijtmans L. A. et al. (1996) Am J Hum Genet 58: 35 - 41.
Cattaneo M. et al. (1997) Arterioscler Thromb Vasc Biol. 17: 1662-1666.

Coagulation ELITe MGB® Kit
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PROCEDURE LIMITATIONS

Use this product only with whole blood clinical samples collected in EDTA.

There are no data available concerning product performance with DNA extracted from the following clinical samples: saliva.

The results obtained with this product depend on an adequate 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 products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the positive samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations. However, cross-contaminations can be avoided only 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 work clothes 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 special clothing and instruments dedicated to work session setup to avoid false positive results.

An invalid or inconclusive result obtained with this product means that it was not possible to efficiently detect the sample genomic DNA or the dissociation temperatures of alleles. In this case the analysis of sample must be repeated with possible delay in obtaining results.

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target DNA and lead to incorrect results.

As with any other diagnostic medical device, the results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, inconclusive and incorrect results obtained 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.

TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and positive control. Check the volumes of PCR Mix and positive control.
Positive control degradation.	Use a new aliquot of positive control.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and negative control. Check the volumes of PCR Mix and negative control.
Contamination of the negative control	Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the Extraction Area, of Racks or of Inventory Block	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid or Inconclusive Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
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.
DNA quantity not sufficient in the sample.	Repeat the extraction and amplification with a new aliquot of the sample in a “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Error 30103	
Possible causes	**Solutions**
Too high concentration of the target in the sample.	Repeat the amplification reaction of the sample with a 1:10 dilution of the eluted sample in water for molecular biology in a session in "PCR Only" mode.

TH Error, SDM error, Ct error	
Possible causes	**Solutions**
Sample with anomalous plot shape.	Repeat the amplification reaction of the sample with a 1:10 dilution of the eluted sample in water for molecular biology in a session in "PCR Only" mode.

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SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostics 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.
	Attention, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

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 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 without undue delay.

NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and sold under license agreements entered into between ELITechGroup S.p.A. and its affiliates and Thermo Fisher Scientific. The purchase price of this product includes non-transferable rights, limited to using only this quantity of product exclusively for the buyer's activities directly related to human diagnostics. For information on license to purchase this product for purposes other than those stated above, contact the Licensing Department, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

ELITe MGB® detection reagents are covered by one or more of U.S. Patents Nos numbers 6972339, 7112684, 7319022, 7348146, 7381818, 7541454, 7582739, 7601851, 7671218, 7718374, 7723038, 7759126, 7767834, 7851606, 8008522, 8067177, 8163910, 8389745, 8569516, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1430147, 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

ELITe InGenius® and ELITe BeGenius® are technology is covered by patents or is the subject of patent applications.

This limited license permits the person or legal entity to which this product has been provided to use the product, and the data generated by use of the product, only for human diagnostics. Neither ELITechGroup S.p.A. nor its licensors grants any other licenses, expressed or implied for any other purposes.

Coagulation ELITe MGB® kit used with ELITe InGenius and ELITe BeGenius

Ref: RTSD00ING

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

A.Intended use

The product **Coagulation ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as qualitative nucleic acids Real-Time PCR assay for the allelic discrimination of the following three loci in human genomic DNA samples extracted from clinical specimens:

- coagulation Factor V gene, single nucleotide polymorphism (SNP) G1691A (Leiden),

- coagulation Factor II gene, SNP G20210A,

- 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, SNP C677T.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®**, automated and integrated instruments for extraction, Real-Time PCR and results interpretation, using human specimens of whole blood collected in EDTA.

The product is intended for use as an aid in assessing the risk of deep vein thrombosis in patients suspected of having coagulation disorders and at risk of deep vein thrombosis.

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

B. Amplified sequence

Target	Gene	Fluorophore
SNP G1691A (Leiden)	Factor V	AP639
SNP G20210A	Factor II	FAM
SNP C677T	MTHFR	AP593
Internal Control	Human beta Globin gene	AP525

C. Validated matrix

Whole Blood collected in EDTA.

D. Kit content

52M PCR Mix	
 X 8	Ready-to-use PCR Master Mix 8 tubes of 280 µL 96 reactions per kit 7 freeze-thaw cycles per tube

Storage temperature: - 20°C or below

E. Material required not provided in the kit

- **ELITe InGenius** instrument: INT030
- **ELITe BeGenius** instrument: INT040
- **ELITe InGenius SP200** extraction cartridge: INT032SP200
- **ELITe InGenius PCR Cassette** amplification cartridge: INT035PCR
- **ELITe InGenius SP200 Consumable Set** consumable for extraction: INT032CS
- **Coagulation - ELITe Positive Control:** CTRD00ING
- **ELITe InGenius Waste Box:** F2102-000
- **300 µL Filter Tips Axygen:** TF-350-L-R-S with ELITe InGenius only
- **1000 µL Filter Tips Tecan:** 30180118 with ELITe BeGenius only

F. Protocol

Sample volume	200 µL
Total eluate volume	200 µL
PCR eluate input volume	20 µL
Q-PCR Mix volume	20 µL
Unit of quantitative result	Not Applicable
Frequency of controls	15 days
Frequency of calibration	Not Applicable

G. Performance

Target	Limit of Detection	Total Diagnostic Agreement
Factor V SNP G1691A	**70ng DNA/reaction**	**100%**
Factor II SNP G20210A	**70ng DNA/reaction**	**100%**
MTHFR SNP C677T	**70ng DNA/reaction**	**100%**

H. Procedures ELITe InGenius

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

Before analysis

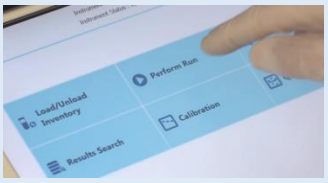
1. Switch on ELITe InGenius
Identification with username and password
Select the mode "Closed"

2. Verify controls: 52M positive and negative controls in the "Control menu"
N.B: Both have been run, approved and not expired

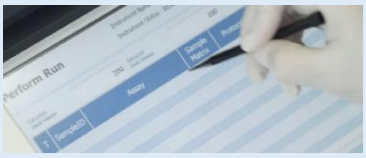
3. Thaw the 52M-PCR-Mix tube
Vortex gently
Spin down 5 sec

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen



2. Verify the extraction volume:
Input: "200 µL", eluate: "200 µL"

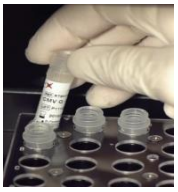


3. Scan the sample barcodes with hand-held barcode reader or type the sample ID

4. Select the "Assay protocol" of interest

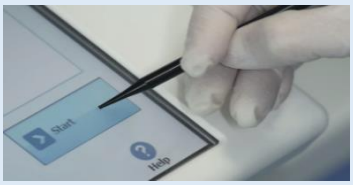
5. Select the sample position:
Primary tube or extraction tube

6. Load the PCR Mix in the inventory block



7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, extraction tube and primary sample racks

8. Close the door
Start the run



9. View, approve and store the results

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above

5. Select the protocol "PCR only" and set the sample position "Extra tube"

6. Load the extracted nucleic acid tubes in the Elution tubes rack

7. Load the PCR cassette rack
Load the PCR Mix in the inventory block

8. Close the door
Start the run

9. View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” and set the sample position: Primary tube or Secondary tube	**6.** Load: Extraction cartridge, Elution tube, Tip cassette, extraction tube and primary sample racks
7. Close the door Start the run	**8.** Archive the eluate sample	

I. Procedures ELITe BeGenius

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

Before analysis

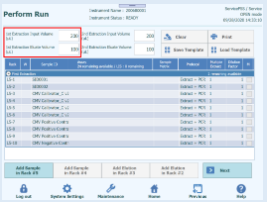
1. Switch on ELITe BeGenius. Log in with username and password. Select the mode “Closed”.	**2.** Verify controls: 52M Positive Control and 52M Negative Control in the “Controls” menu. *Note:* Both must have been run, approved and not expired.	**3.** Thaw the 52M PCR Mix tube. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extraction + PCR

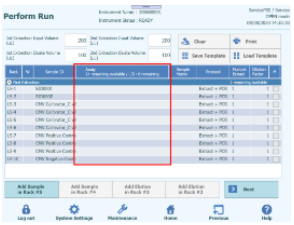
1. Select “Perform Run” on the touch screen and then click on the run mode «Extraction and PCR»

2. Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active

3. Verify the extraction volumes: Input: “200 µL”, Eluate: “200 µL”

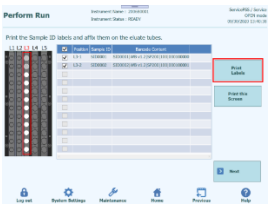


4. Select the “Assay protocol” of interest



Note: if a second extraction is performed repeat steps from 2 to 4

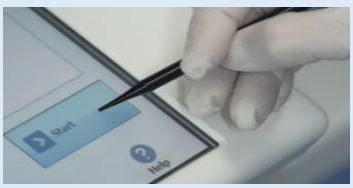
5. Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area



6. Load the Q-PCR-Mix in Reagent Rack and insert it in the cooling area

7. Load: Filter Tips, Extraction rack, and PCR rack

8. Close the door. Start the run



9. View, approve and store the results

Procedure 2 - PCR only

1. Select “Perform Run” on the touch screen and the click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid barcoded tubes in the Elution Rack and insert it in the cooling area	**3.** Select the “Assay protocol” of interest
4. Load the Q-PCR-Mix in Reagent Rack and insert it in the cooling area Load filter tips and the PCR rack	**5.** Close the door. Start the run	**6.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol “Extraction Only” in the Assay Protocol selection screen.	**6.** Load : Filter Tips and the Extraction Rack
7. Close the door Start the run	**8.** Archive the eluate sample	