

A. Intended use

The «SARS-CoV-2 Extended ELITE MGB® Kit» product is part of a qualitative multiplex nucleic acids reverse transcription and amplification and melting curve analysis assay, for the detection and discrimination of the mutations L452R, L452Q, E484K, E484Q, E484A, Q498R and N501Y of the S gene of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in clinical samples from subjects infected by the virus.



The assay is able to detect the mutations associated with the following variants: the Alpha variant (UK), lineage B.1.1.7, the Beta variant (South Africa), lineage B.1.351, the Gamma variant (Brazil), lineage P.1, the Delta variant (India), lineage B.1.617.2, the Kappa variant (India), lineage B.1.617.1, the Epsilon variant (USA), lineage B.1.427/9, the Eta variant (Multiple countries), lineage B.1.525, the Iota variant (USA), lineage B.1.526, the Lambda variant (Perù), lineage C.37 and the Mu variant (Colombia), lineage B.1.621 and the Omicron variant (Multiple countries), lineage B.1.1.529.

The product is used as a reflex test to identify the possible presence of the L452R and L452Q, N501Y and Q498R + N501Y, E484K, E484Q and E484A mutations of the SARS-Cov-2 S gene, in samples already diagnosed as positive for SARS-CoV-2. The test is intended for Research Use Only. Before use, the laboratory should validate the whole process.



B. Matrices

Respiratory Swabs	Copan Ref.	Description
	360C or 305C	UTM kit

D. SARS-CoV-2 Extended ELITE MGB® KIT (RTS172ING) content

CoV-2 Extended PCR Mix (Neutral cap)	RT EnzymeMix (Black cap)	Maximum Shelf-life: 12 Months
 X 2	 X 2	Storage temp.: below - 20 °C
2 tubes of 1200 µL 96 reactions per kit 6 freeze-thaw cycles	2 tubes of 20 µL 96 reactions per kit 6 freeze-thaw cycles	Prepare the complete reaction mixture in a 2 mL Sarstedt tube (Ref. 72694005, not provided)

E. SARS-CoV-2 Extended - ELITE Positive Control (CTR172ING) content

CoV-2 Ext Wild Type Positive Control (Black cap)	CoV-2 Ext Mutant Positive Control (Red cap)	Maximum Shelf-life: 24 Months
 X 2	 X 2	Storage temp.: below - 20 °C
2 tubes of 160 µL 4 sessions / tube 4 freeze-thaw cycles	2 tubes of 160 µL 4 sessions / tube 4 freeze-thaw cycles	

F. Complete reaction mixture Preparation

1. Thaw the CoV-2 Extended PCR Mix and RT EnzymeMix tubes and SARS-CoV-2 Extended - ELITE Positive Control,
2. Mix gently and spin-down,
3. Reconstitute the complete reaction mixture in a 2 mL Sarstedt tube (Ref. 72694005) as shown below:

Reagent	1 Test	Samples N	Total reactions
CoV-2 Extended PCR Mix	20 µL	1 to 5	N + 1
RT EnzymeMix	0.3 µL	6 to 12	N + 2
		13 to 24	N + 3
		25 to 48	N + 4

4. Mix gently and spin-down.
5. Pipet 20 µL of complete reaction mixture in all microplate wells or Strip Tubes in use,
6. Add 10 µL of extracted RNA in sample wells, 10 µL of molecular grade water in Negative Control well and 10 µL of each Positive Control in control wells. **Note:** Each one has to be mixed by pipetting 3 times into the reaction mixture,

7. Seal the microplate with the amplification sealing sheet,
8. Transfer the microplate in the thermocycler and start.

G. Result Interpretation

Sample results:							
Result of sample run	Interpretation						
SARS-CoV-2 452L wild type	The RNA of SARS-CoV-2 452L wild type was detected in the sample						
SARS-CoV-2 mutation L452R detected	The RNA of SARS-CoV-2 452R mutant was detected in the sample						
SARS-CoV-2 mutation L452Q detected	The RNA of SARS-CoV-2 452Q mutant was detected in the sample						
SARS-CoV-2 484E wild type	The RNA of SARS-CoV-2 484E wild type was detected in the sample						
SARS-CoV-2 mutation E484K detected	The RNA of SARS-CoV-2 484K mutant was detected in the sample						
SARS-CoV-2 mutation E484Q detected	The RNA of SARS-CoV-2 484Q mutant was detected in the sample						
SARS-CoV-2 mutation E484A detected	The RNA of SARS-CoV-2 484A mutant was detected in the sample						
SARS-CoV-2 501N wild type	The RNA of SARS-CoV-2 501N wild type was detected in the sample						
SARS-CoV-2 mutation N501Y detected	The RNA of SARS-CoV-2 501Y mutant was detected in the sample						
SARS-CoV-2 presumptive Q498R+N501Y detected	The RNA of SARS-CoV-2 presumptive 498R+501Y was detected in the sample						
SARS-CoV-2 452 NOT amplified	The RNA of SARS-CoV-2 452 was not detected in the sample						
SARS-CoV-2 484 NOT amplified	The RNA of SARS-CoV-2 484 was not detected in the sample						
SARS-CoV-2 501 NOT amplified	The RNA of SARS-CoV-2 501 was not detected in the sample						
Invalid	Not valid result caused by Internal Control failure						
Sample Ct and Tm ranges:							
Ct 452	Ct 484	Ct 501	Tm 452	Tm 484	Tm 501	Ct RNase P	Result
Ct < 45			59.6 – 63.6			+/-	SARS-CoV-2 452L wild type
Ct < 45			52.8 – 55.2			+/-	SARS-CoV-2 mutation L452R detected
Ct < 45			55.3 – 57.7			+/-	SARS-CoV-2 mutation L452Q detected
	Ct < 45			64.4 - 68.1		+/-	SARS-CoV-2 484E wild type
	Ct < 45			58.5 – 61.3		+/-	SARS-CoV-2 mutation E484K detected
	Ct < 45			55.7 – 58.4		+/-	SARS-CoV-2 mutation E484Q detected
	Ct < 45			62.0 – 64.3		+/-	SARS-CoV-2 mutation E484A detected
		Ct < 45			54.7 - 58.4	+/-	SARS-CoV-2 501N wild type
		Ct < 45			59.6 - 65.6	+/-	SARS-CoV-2 mutation N501Y detected
					52.9 – 54.6		SARS-CoV-2 presumptive Q498R+N501Y detected
Ct > 45	Ct > 45	Ct > 45				Ct < 35	SARS-CoV-2 NOT amplified
Ct > 45	Ct > 45	Ct > 45				Ct > 35	Invalid

Positive Control Ct and Tm limits:

Controls	Target	Ct	452 Tm	484 Tm	501 Tm	Result
CoV-2 Ext Wild Type Positive Control	L452 wt	Ct < 31				Positive Control Valid
	E484 wt	Ct < 31	59.6 – 63.6	64.4 - 68.1	54.7 - 58.4	
	N501 wt	Ct < 31				
CoV-2 Ext Mutant Positive Control	452R mut	Ct < 33				Positive Control Valid
	484K mut	Ct < 32	52.8 – 55.2	58.5 – 61.3	59.6 - 65.6	
	501Y mut	Ct < 30				
Negative Control	-	Ct > 45	-	-	-	Negative Control Valid

Note: The Ct and Tm Limits were defined on Bio-Rad CFX96 Dx instrument. The Ct and Tm Limits for ABI 7500 Fast Dx / 7500 / QuantStudio5 Dx shall be verified by users.

H. Samples

1. Sample inactivation is not required (ref. WHO guideline). The sample can be pretreated with a denaturant solution or by a heat incubation under a Biosafety cabinet of class II (BSC2). In case of this procedure, dispense 200 µL of sample into the tubes and no more than 200 µL of denaturant buffer. Note: This procedure is an off-label protocol and needs to be validated before use (ref WHO guidelines).
2. Handle and dispose of all biological samples as if they were able to transmit infectious agents even if the sample is inactivated.
3. Eluates obtained with extraction in association with SARS-CoV-2 ELITE MGB® Kit (Ref. RTS170ING) or SARS-CoV-2 PLUS ELITE MGB Kit (Ref. RTS180ING) can be used.
4. High-medium viral titre for SARS- CoV-2 allows to obtain better results.

I. References

- ECDC Reference: "Assessing SARS-CoV-2 circulation, variants of concern, non-pharmaceutical interventions and vaccine rollout in the EU/EEA, 15th update" June 2021.
- WHO Reference: "Laboratory testing for coronavirus disease (COVID-19) in suspected human cases, 2 March 2020".

J. Semi-automated extractions compatibility

ELITechGroup ELITE GALAXY

ELITE GALAXY is a closed instrument, but an open protocol is ready for use with SARS-CoV-2 Extended ELITE MGB® Kit. Please refer to your local ELITechGroup support to ask the required protocol to be upload into the instrument.

Sample preparation

ELITE GALAXY – Extraction kit (Ref. INT021EX)	
Sample volume processed:	300 µL
Sample dead volume (2mL tubes):	100 µL
Sample dead volume (13x75 tubes)	300 µL
Sample dead volume (16x100 tubes)	600 µL
Total elution volume:	100 µL

PCR setup

Master Mix volume:	20 µL
Master Mix Dead volume:	45 µL
Eluate volume:	10 µL
Controls dead volume:	30 µL

Qiagen QIASymphony

QIAGEN QIASymphony is a closed instrument that allows the usage of open protocols. Please refer to your local Qiagen support to ask an offer to provide you the required protocol.

Sample preparation

QIASymphony - DSP Virus/Pathogen Midi kit	
Sample volume processed:	400 µL
Total elution volume:	85 µL

PCR setup

Master Mix volume:	20 µL
Master Mix Dead volume:	60 µL
Eluate volume:	10 µL
Controls dead volume:	30 µL

Abbott m2000sp

Abbott m2000sp is a closed instrument that allows the usage of open protocols. Please refer to your local Abbott support to ask an offer to provide you the required protocol. It can be needed enabling the fluorescence channels.

Sample preparation

Abbott m2000sp - mSample Preparation Systems DNA (CE) or mSample Preparation Systems RNA	
Sample volume processed:	400 µL – 600 µL
Total elution volume:	100 µL

Note: m2000sp requires to extract the positive and negative controls. Substitute the controls with water and at the end remove them from the elution plate.

PCR setup

Master Mix volume:	20 µL
Master Mix Dead volume:	100 µL
Eluate volume:	10 µL
Controls:	10 µL - Manual input

Note: Positive and negative controls are manually dispensed in the PCR plate after the automatic dispensation of the Master Mix and the patient samples eluates.

Siemens Versant kPCR

Siemens Versant kPCR allows the usage of open protocols. Please refer to your local Siemens support to ask an offer to provide you the required protocol.

Sample preparation

Versant kPCR - Sample Preparation 1.0 Reagents Kit	
Sample volume processed:	400 µL
Total elution volume:	100 µL

PCR setup

Master Mix volume:	20 µL
Master Mix Dead volume:	45 µL
Eluate volume:	10 µL
Controls dead volume:	30 µL

MGISP-960

MGI Tech MGISP-960 is an automatic system for DNA /RNA extraction and PCR – setup. The **Biorad CFX-96** or **Thermo Fisher 7500 Fast DX / 7500** are used in association with this system. Please refer to your local ELITechGroup support to ask the required protocol.

Sample preparation

MGIeasy Magnetic Beads Virus DNA/RNA Extraction kit (EuroClone code 909-1000021043)	
Sample volume processed:	180 µL
Dispense manually the sample in the "processing plate"	
Total elution volume:	30 -50 µL

PCR setup

Master Mix volume:	20 µL
<i>If 1 plate is working: Dispense 240 µL of Master Mix for each well in the first column (A)</i>	
<i>If 2 plates are working: Dispense 480 µL of Master Mix for each well in the first column (A)</i>	
Eluate volume:	10 µL
Controls volume:	10 µL

GenePure PRO-Bioer

GenePure Pro-Bioer is an automatic system for DNA /RNA extraction. The ELITE InGenius® is used in association with this system. Please refer to your local ELITechGroup support to ask the required protocol.

Sample preparation

MagaBio Plus Virus DNA/RNA purification Kit (code 909-BSC71S1E)	
Sample volume processed:	300 µL
Proteinase K (PK):	10 µL
<i>Dispense manually firstly the sample and then PK in the deep-well</i>	
Total elution volume:	70 µL

PCR setup

Master Mix volume:	20 µL
Eluate volume:	10 µL
Controls volume:	10 µL

K. Real Time PCR thermal cycler compatibility

Reaction Volume

30 µL

Bio-Rad CFX96 Dx

Verify before use.

Amplified sequence and Channels setting

Target	Gene	Channels	Threshold
Target 1	S gene, codon 452	Cy5	Auto
Target 2	S gene, codon 484	FAM	Auto
Target 3	S gene, codon 501	ROX	Auto
IC	Rnase P gene	VIC	Auto

Baseline threshold settings

Setting	Value
Baseline	Auto

Thermal Profile

	Description	Temperature	Time
Hold	Reverse Transcription	45 °C	20:00
Hold 2	Pre-Denaturation	95 °C	02:00
Cycling 44 cycles	Denaturation	95 °C	00:10
	Annealing (+Plate Read)	56 °C	00:20 (+00:10)
	Extension	72 °C	00:20
Dissociation Stage	Description	Temperature	Time
		95 °C	00:15
		45 °C	01:00
		45 °C	00:01
		80 °C*	

*increment 0.5 °C

ABI 7500 Fast Dx / 7500 / QS-5 Dx

These settings could be theoretically compatible also with other similar instruments like the Quant Studio version 6/7 and 7500. Verify before use.

Amplified sequence and Channels setting

7500 Fast Dx / 7500

Target	Gene	Channels	Threshold*	Quencher
Target 1	S gene, codon 452	Cy5	10000	none
Target 2	S gene, codon 484	FAM	10000	none
Target 3	S gene, codon 501	ROX	5000	none
IC	Rnase P gene	VIC	50000	none

*TBD for 7500 instrument

Thermal Profile

	Description	Temperature	Time
Hold	Reverse Transcription	45 °C	20:00
Hold 2	Pre-Denaturation	95 °C	02:00
Cycling 45 cycles	Denaturation	95 °C	00:10
	Annealing (Plate Read)	56 °C	00:30
	Extension	72 °C	00:20
Dissociation Stage	Description	Temperature	Time
		95 °C	00:15
		45 °C	01:00
		80 °C	00:15
		60 °C^	00:15

For 7500 Fast Dx and QS5 Dx set the Run Mode as Fast 7500

^For QS-5 DX the step at 60° C has not be set

Interpretation settings

Setting	Value	Value
Passive	7500FDX	QS-5 Dx
Reference	none	none
Baseline*	Manual (6-15)	AUTO

*TBD for the other instruments

Note: For QS-5 DX flag on all Melt Curve Filter in Optical filter settings to activate dissociation stage.