

SARS-CoV-2 Extended ELITE MGB® Kit (Cod.: RTS172ING)
SARS-CoV-2 Extended - ELITE Positive Control (Cod.: CTR172ING)
 used in association with ELITE InGenius®



This document is available in English and Italian.
 For further information please refer to: ingenius.support@elitegroup.com



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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 P1, 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. Amplified sequence

Target	Gene	Fluorophore	Channel
Target 1	S gene, codon 484	FAM	484 (Ch1)
Target 2	S gene, codon 501	AP593	501 (Ch4)
Target 3	S gene, codon 452	AP639	452 (Ch5)
Internal Control (endogenous)	RNase P gene	AP525	IC (Ch3)

C. Matrices



› Respiratory Swabs

Note: Transfer 200 µL of sample from the Swab tube into the extraction tube. Do not insert the primary tube directly into the instrument to perform the run.

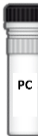
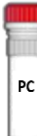
D. Tube type collection

Copan Ref.	Description
360C or 305C	UTM kit

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

CoV-2 Extended PCR Mix (Neutral cap)	RT EnzymeMix (Black cap)	
 X 2	 X 2	Maximum Shelf-life: 12 Months
		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 tube (Sarstedt Ref. 72694005, not provided)

F. 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)	
 X 2	 X 2	Maximum Shelf-life: 24 Months
		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	

G. Material required not provided in the kit

› ELITE InGenius instrument:	INT030
› ELITE InGenius SP200 Extraction Cartridge:	INT032SP200
› ELITE InGenius PCR Cassette:	INT035PCR
› ELITE InGenius SP200 Consumable Set:	INT032CS
› ELITE InGenius Waste Box:	F2102-000
› 300 µL Filter Tips Axygen:	TF-350-L-R-S

H. ELITE InGenius® Protocol

Protocol	Volume
Sample	200 µL
Total eluate	100 µL
PCR eluate input	10 µL
Complete PCR Mix	20 µL
Control Frequency	15 days

I. Result Interpretation

Sample results:

Result of sample run	Interpretation
452: RNA Detected. 452L Wild Type	The RNA of SARS-CoV-2 452L wild type was detected in the sample
452: RNA Detected. 452R Mutant	The RNA of SARS-CoV-2 452R mutant was detected in the sample
452: RNA Detected. 452Q Mutant	The RNA of SARS-CoV-2 452Q mutant was detected in the sample
452: RNA Detected	The RNA of SARS-CoV-2 was detected in the sample, but 452 typing was not feasible
484: RNA Detected. 484E Wild Type	The RNA of SARS-CoV-2 484E wild type was detected in the sample
484: RNA Detected. 484K Mutant	The RNA of SARS-CoV-2 484K mutant was detected in the sample
484: RNA Detected. 484A Mutant	The RNA of SARS-CoV-2 484A mutant was detected in the sample
484: RNA Detected	The RNA of SARS-CoV-2 was detected in the sample, but 484 typing was not feasible. If the T _m falls within 57.3 – 59.3 °C the mutation E484Q was detected
501: RNA Detected. 501N Wild Type if 484A Not Detected	The RNA of SARS-CoV-2 501N wild type was detected in the sample if the 484A mutation was not detected
501: RNA Detected. 501Y Mutant	The RNA of SARS-CoV-2 501Y mutant was detected in the sample
501: RNA Detected. 498R+501Y Mutant if 484A Detected	The RNA of SARS-CoV-2 498R+501Y mutant was detected in the sample if the 484A mutation was detected
501: RNA Detected	The RNA of SARS-CoV-2 was detected in the sample, but 501 typing was not feasible
452: RNA Not Detected or below the LoD	The RNA of SARS-CoV-2 452 was not detected in the sample
484: RNA Not Detected or below the LoD	The RNA of SARS-CoV-2 484 was not detected in the sample
501: RNA Not Detected or below the LoD	The RNA of SARS-CoV-2 501 was not detected in the sample
Invalid – Retest sample	Not valid result caused by Internal Control failure
Typing not determined	The RNA of SARS-CoV-2 is detected in the sample, but it is not possible to calculate the T _m or T _m value is out of the intervals determined for typing

Sample Ct and T_m ranges:

Ct 452	Ct 484	Ct 501	T _m 452	T _m 484	T _m 501	Ct RNase P	Result
Det.	-	-	61.4 – 64.4	-	-	+/-	SARS-CoV-2 452L wild type
Det.	-	-	54.2 – 57.2	-	-	+/-	SARS-CoV-2 mutation L452R detected
Det.	-	-	57.3 – 59.7	-	-	+/-	SARS-CoV-2 mutation L452Q detected
-	Det.	-	-	66.0 – 68.6	-	+/-	SARS-CoV-2 484E wild type
-	Det.	-	-	59.4 – 61.9	-	+/-	SARS-CoV-2 mutation E484K detected
-	Det.	-	-	63.1 – 65.9	-	+/-	SARS-CoV-2 mutation E484A detected
-	-	Det.	-	-	56.8 – 58.6	+/-	SARS-CoV-2 501N wild type if 484A Not Detected
-	-	Det.	-	-	62.6 – 65.6	+/-	SARS-CoV-2 mutation N501Y detected
-	-	Det.	-	-	54.3 – 56.7	+/-	SARS-CoV-2 Q498R+N501Y detected if 484A Detected
Undet.	Undet.	Undet.	-	-	-	Ct < 35	SARS-CoV-2 NOT amplified
Undet.	Undet.	Undet.	-	-	-	Ct > 35	Invalid

Note: For the target 484 if T_m value falls within the T_m range 57.3 - 59.3 °C, the 484Q mutation is detected in the sample.

Positive Control Ct and Tm limits:

Controls	Target	Ct	452 Tm	484 Tm	501 Tm	Result
CoV-2 Ext Wild Type Positive Control	452L wt	Ct < 29	61.5 – 64.5	65.9 – 68.8	56.5 - 58.7	Positive Control Valid
	484E wt	Ct < 31				
	501N wt	Ct < 36				
CoV-2 Ext Mutant Positive Control	452R mut	Ct < 31	54.3 – 57.3	59.4 – 61.9	62.6 - 65.6	Positive Control Valid
	484K mut	Ct < 32				
	501Y mut	Ct < 31				
Negative Control	452	Undet.		-		Negative Control Valid
	484	Undet.		-		
	501	Undet.		-		

J. Sample Preparation

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.

K. Procedures

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

Before analysis

- | | | |
|--|---|---|
| <ol style="list-style-type: none"> 1. Switch on ELITE InGenius Identification with username and password
Select the mode “Open” | <ol style="list-style-type: none"> 2. Verify controls in the “Control menu”:
CoV-2 EXT Positive Control,
CoV-2 EXT Negative Control,
Note: All have to be run, approved and not expired | <ol style="list-style-type: none"> 3. Thaw the CoV-2 Extended PCR Mix and RT EnzymeMix tubes.
Vortex gently and spin down 5 sec.
Keep RT EnzymeMix on ice. |
|--|---|---|

4. Reconstitute the complete reaction mixture in a 2 mL Sarstedt tube (Ref.72694005) as shown in the table below:
5. Vortex gently and spin down 5 sec.
Keep the complete reaction mixture on ice.
Do not expose to direct light.

Number of samples (N)	CoV-2 Extended PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

Procedure 1 - Complete run: Extraction + PCR

- | | | |
|---|---|---|
| <ol style="list-style-type: none"> 1. Select “Perform Run” on the touch screen | <ol style="list-style-type: none"> 2. Verify the extraction volumes:
Input: “200 µL”, Elute: “100 µL” | <ol style="list-style-type: none"> 3. Scan the sample barcodes with barcode reader or type the sample ID |
| <ol style="list-style-type: none"> 4. Select the “Assay Protocol” of interest: SARS-CoV-2 EXT ELITE_Open_200_100 | <ol style="list-style-type: none"> 5. Select the mode “Extract + PCR”. Set the sample position: “Extraction tube”.
Note: 200 µL of sample must be transferred into Extraction tube. For samples see C and D | <ol style="list-style-type: none"> 6. Load the complete reaction mixture in the inventory block |
| <ol style="list-style-type: none"> 7. Load the PCR cassette, Extraction cartridge, Elution tubes, Tips and Extraction tube racks | <ol style="list-style-type: none"> 8. Close the door.
Start the run. | <ol style="list-style-type: none"> 9. View, approve and store the results |

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above	5. Select the protocol "PCR only" and set the sample position: "Elution tube"	6. Load the extracted nucleic acid tubes in the Elution tubes rack
7. Load the complete reaction mixture in the inventory block Load the PCR cassette, Elution tubes, Tip racks	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 mode: "Extraction Only" Set the sample position: "Extraction tube" Note: 200 µL of sample must be transferred into Extraction tube	6. Load the Extraction cartridge, Elution tubes, Tips and Extraction tube racks.
7. Close the door. Start the run.	8. Archive the eluate samples.	

L. 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".