TIB MOLBIOL

Instructions For Use

VirSNiP SARS-CoV-2 Spike E484K

530

Cat.-No. 53-0789-96 Roche SAP n° 09 429 549 001

Kit with reagents for 96 PCR reactions 20 µl for genotyping of SARS-CoV-2 RNA [lyophilized]

1. Content, Storage and Expiry

Storage at Arrival:

1 Vial yellow cap 96 reactions SARS CoV (lyophilized)
1 Mixed positive control

Store cooled or at ambient temperature
Do not freeze the lyophilized reagents.

- Kits are stable for one year after production (store 4°C to 25°C in the dark). See lot-specific expiry date.
- Reconstituted reagents are stable for two weeks if stored protected from light and cooled (2°C to 8°C).
- Dissolved reagent can be stored long-term if frozen (-15°C to -25°C). Avoid multiple freeze-thaw cycles.
- Reconstituted positive controls must be stored frozen. Minimize freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex RNA Virus Master Cat.-No. 06 754 155 001
or 1-step RT polymerase 90-9999-96

3. Introduction

Hundreds of thousands of isolates have been sequenced since the SARS-CoV-2 genome was published (MN908947). The *'variants of concern'* share the 501Y mutation; UK can be identified via the deletion, the South African variant by the 484 mutation; meantime more strains have the 484K mutation :

Spike Prot. Variation	Genetic Variation	UK B.1.1.7	UK B.1.525	ZA B.1.351	Brazil P.1	Brazil P.2	DK mink Clust V	Function, Effect	Single assay
A67V	C21762T		x						
del HV69/70	del21765-770	x	x				x	evasion of immune response	53-0781
K417N	G22813T			x	417T			RBD (ACE binding domain)	53-0787
N439K	C22879A							ACE binding, immune escape	53-0788
Y453F	A22920T						x	RBD (weaker ACE binding)	53-0783
E484K	G23012A		x	x	x	x		RBD (ACE binding domain)	53-0789
N501Y	A23063T	x		x	x			RBD (stronger ACE binding)	53-0780
A570D	C23271A	x							53-0791
D614G	A23403G	x	x	x	x	x	x	RBD (stronger ACE binding)	53-0782
P681H	C23604A	x						furin cleavage site	53-0786
V1176F	G25088T				x	x		increased mortality	53-0784

4. Description

A 160 bp PCR long fragment is amplified and analyzed with a melting curve using a 484K-specific probe. For using Roche polymerase the amplification of both variants 484E and 484K is not visible.

5. Specification

Sensitivity better than 50 copies viral RNA UK strain B.1.1.7 (50% signal compared to 4,000 copies).

6. Sample Material and Extraction

Coronaviruses affect normally the lower respiratory system, but SARS-CoV-2 is found also in nose and throat. Typical clinical samples are throat and nasopharyngeal swabs, sputum, saliva or gargle solution. Product tested with heat-treated gargle solution. For RNA extraction see manufacturer's kit instructions.

7. Material Safety Data (MSDS)

This product is not hazardous (according to regulation (EC) No 1272/2008), not toxic, not IATA-restricted. Not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and EU Directives (EC) No 1907/2006 and (EC) No 2015/830 any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a MSDS.

8. Instructions for Use

Instruction for Roche 480 instruments. Capillary LightCycler®, LightCycler® 96, MyGo and BioRad CFX96 instruments give similar results (FAM channel). For other instruments use SYBR Green melting option.

8.1. Programming Roche 480 Instruments (Standard ModularDx Program)

Detection Format 530 Channel **Set Quant Factor 10, Max Integration Time 1 sec**
LightCycler® 480 Instrument: 483-533
LightCycler® 480 II Instrument: 465-510
cobas z 480 Analyzer (open channel): 465-510

Program Step:	***RT Step***	***Denaturation***	***Cycling***			***Cooling***
Parameter						
Analysis Mode	***None***	None	Quantification mode			None
Cycles	*1*	1	40-45			1
Target [°C]	55	95	95	60	72*	40
Hold [hh:mm:ss]	00:05:00	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	**Single**	None	None

* 72°C step can be skipped. 95°C can be cut to 3 s, 60°C to 12 s. RT and Den to 3 min (total time 45 min) Table 1

8.1.1. Melting Analysis (may be added or programmed as second run)

Detection Format Hydrolysis Probe or SimpleProbe
LightCycler® 480 Instrument: 483-533
LightCycler® 480 II Instrument: 465-510
cobas z 480 Analyzer (open channel): 465-510

Program Step:	***Melting***			***Cooling***
Parameter				
Analysis Mode	Melting Curves mode			None
Cycles	1			
Target [°C]	95	40	75	40
Hold [hh:mm:ss]	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate [°C/s]	4.4	1,5	-	1.5
Acquisition Mode	-	-	**Continuous**	
Acquisitions [per °C]	-	-	3**	None

Table 2

** Melting slope shall be 0.19 to 0.29°C per second. If reading more channels reduce the number of acquisitions/sec.

8.2. Experimental Protocol

- **Sample material:** Use aqueous nucleic acid preparations
- **Negative control:** Always run at least one no-template control (NTC) - replace the template NA with water.
- **Positive control:** Run a positive control - replace the template NA with the provided Positive Control.

For an increased sensitivity use 10 µl nucleic acid per 20 µl reaction.
Product tested for 10 µl reaction volume (192 reactions).

8.2.1. Preparation of Parameter-Specific Reagents (PSR, 96 reactions):

The reagent vial with the **yellow** cap contains the primers and probe to run 96+ PCR reactions.

Check for the orange pellet, then **add 50 µl** PCR-grade water, mix (vortex) and spin down.
For robotic pipetting the volume can be extended to 55 µl (signals will decrease by 10-20%).

►**Use 0.5 µl** reagent per 20 µl PCR reaction.

8.2.2. Preparation of the Positive Control

Add 160 µl PCR-grade water to the vial with the **black** cap, if using 10 µl sample volume add **320 µl**.
Mix by pipetting up and down 10 times. If vortexing spin down to collect the solution.
Store dissolved controls frozen.

Notes: Opening this vial may cause contamination of the workspace. Pulse spin vial prior to opening.

►**Use 5 µl** positive control (≈ Cp 27-30) for a 20 µl PCR reaction (10 µl if using 10 µl sample volume).

8.2.3. Preparation of the Reaction Mix

Multiply volumes by the number of reactions plus controls and one reserve, prepare in a cooled tube:

LightCycler® Multiplex RNA Virus Master		1-step RT Polymerase 90-9999-96	
10.4 µl	**Water**, PCR-grade	**Water**, PCR-grade	4.4 µl
0.5 µl	**This reagent (PSR)**	**This reagent (PSR)**	0.5 µl
4.0 µl	**Roche Mastermix**	**TIB Mastermix**	10.0 µl
0.1 µl	**RT Enzym**	-	-
15.0 µl			**15.0 µl**

Table 3

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.

Add 5 µl (10 µl) of sample or control to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.

Start run

9. Typical Results

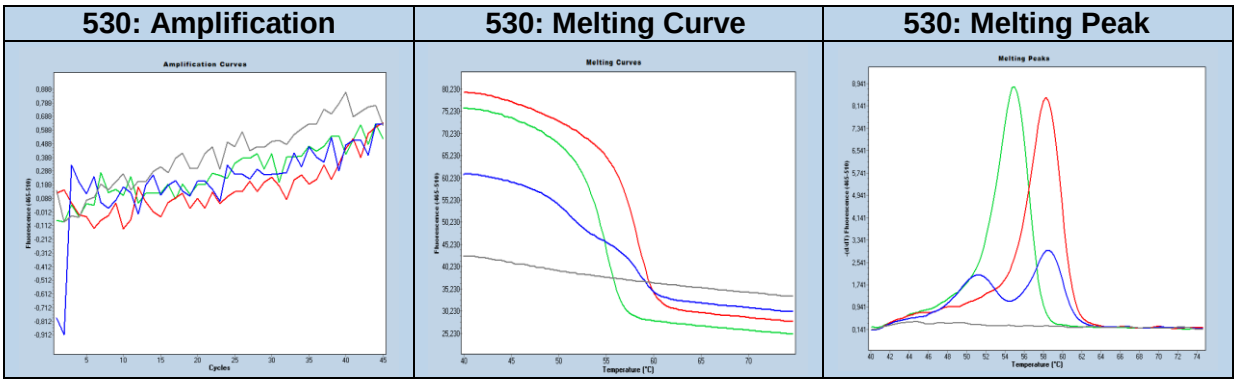

Figure 1. Green 484E, Red mutated, grey NTC **Left** Amplification not visible. **Center** Melting curves. **Right** 484E has a melting point of 55°C (± 2)°C, 484K has a Tm of 58°C (± 2)°C. Positive control is a mixture 484Q and 484K

10. Reading the Results

View results in the 530 channel. Use 'Tm calling'. The negative control (NTC) must show no signal.

Channel 530 Amplification	Channel 530 Melting analysis	Channel 530 NTC Control	Result
Not relevant	Not relevant	**Negative / no peak**	**No virus amplified / not detectable**
Invisible	**Tm ~ 51°C***	**Negative**	**SARS Spike 484Q**
Invisible	**Tm ~ 55°C***	**Negative**	**SARS Spike 484E (not mutated)**
Invisible	**Tm ~ 58°C***	**Negative**	**SARS Spike 484K (e.g. ZA variant)**
Not relevant	Not relevant	**Positive**	**Contamination Repeat experiment**

Tm values shift depending on the instrument, speed of heating, mastermix, salt contents and detection format.
* Temperatures with 1step RT pol. 90-9999-96 are 3-4°C higher.
Single peak with lower Tm values are an indication for the presence of another mutation in the probe region.

The assay detects the genetic situation and not a strain; the correlation to a reference strain describes the most likely assignment for European isolates isolated winter 2020/2021.

11. References

Genomic characterisation of emergent SARS-CoV-2 lineage in UK defined by novel set of spike mutations. Rambaut et al., 2020

www.ecdc.europa.eu/sites/default/files/documents/Detection-and-characterisation-capability-for-SARS-CoV-2-variants-EU%20EEA.pdf

Fast and cost-effective screening for SARS-CoV-2 variants in a routine diagnostic setting. Durner et al,. 2021

Rapid SARS-CoV-2 variants spread detected in France using specific RT-PCR testing. Haim-Boukobza et al., 2021

Rapid detection of SARS-CoV-2 VOC identifying a cluster of B.1.1.28/P.1 2 variant in Br. Col., Canada. Matic et al., 2021

www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/DESH/Bericht_VOC_05022021.pdf?__blob=publicationFile

12. Multiplex PCR Compatibility

This SNP assay can be combined with 51-0776-96 SARS E+N and either EAV spiked extraction control or UBC human mRNA extraction control or with the complete kit 60-0770-96 Sarbecovirus E+N+UBC.

Multiplex PCR and Instrument Compatibility

Color Comp 40-0320 mandatory only for Multiplex PCR using more channels

500	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
	SNP					X	X	X	X	X
SarbecoV	SNP	UBC mRNA				X	X	X		
SarbecoV	SNP				UBC	X	X	X		
SarbecoV	SNP				EAV	X	X	X		
	SNP	SARS N	SARS E		UBC	X	X	X		

Table 3

13. Version History

V210101	Release version	2020-12-31
V210122	8.2.2 Positive control included 8. Short PCR 12. Multiplex	2021-01-22
V210123	8.2.3 Instructions for 1-step RT polymerase 90-9999-96	2021-02-07
V210313	Mutation table	2021-03-13
V210404	Primer moved (better sensitivity), CoA temperatures	2021-03-13

Certificate of Analysis (CoA)

Lot n° **5063**

Expiry : **YYYY-MM-DD**

	484E	484K	484Q		PC	passed
Tm range	54-56°C	56-59°C	49-52°C	**Cp range**	-	
Measured					-	✓
Signal level	2-10	2-10	2-10			
Measured						✓
Negatives	**10/10**					✓

Note: Cp (crossing point) values collected with pDNA (single target PCR). Fluorescence (FL) levels depend on instrument settings and may vary. The Cp values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (ΔCp).

DOM (manufactured): YYYY-MM-DD QC Acceptance: YYYY-MM-DD

We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.

Name(s) :

Name1 *Name2*

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