TIB MOLBIOL

Instructions For Use

VirSNiP SARS-CoV-2 Spike N501Y

530

Cat.-No. 53-0780-96 Roche SAP n° 09 405 577 001

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 Positive control wild type ivRNA (LOW control 30-0780)

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 Roche Cat.-No. 06 754 155 001
or 1-step RT polymerase 90-9999-96

3. Introduction

The SARS-CoV-2 genome was published 11.1.20 (Genbank MN908947). Hundreds of thousand isolates have been sequenced. The UK* strain VUI-202012/01 contains spike variants del69/70, del144, **N501Y**, A570D, P681H, T716I, S982A and D1118H. **501Y** is found in many of the more contagious variants, in particular in B.1.1.7, South African B.1.351 and Brazilian P.1 strains, and is a powerful screening marker.

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

* 501Y samples can be other strains, eg. MT293209 (US). UK must be confirmed by testing for the del69/70, 570D or 681H.

4. Description

A 160 bp PCR long fragment is amplified and analyzed with a melting curve using a 501Y-specific probe. For using Roche polymerase the amplification of isolates containing the 501N variant 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**	2.0
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 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	4.5 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 obtained with Roche Polymerase

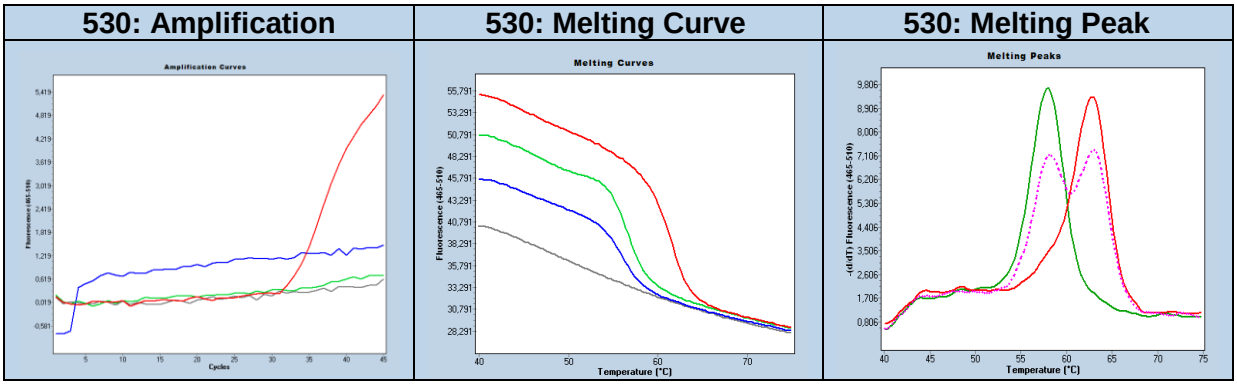

Figure 1. Blue SARS RNA, Green 501N, Red 501Y, Pink pos control. **Left** Only 501Y visible in the amplification. **Center** Melting curves. **Right** 501N has a melting temperature of 56.5 (± 2)°C, 501Y has a Tm of 61.2 (± 2)°C.

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**
Not relevant	**Tm << 55°C***	**Negative**	**Other mutation, eg. T23062C or A23064C**
Invisible	**Tm ~ 56.5°C***	**Negative**	**SARS Spike 501N (not the UK variant)**
Visible	**Tm ~ 61.2°C***	**Negative**	**SARS Spike 501Y (UK or ZA variant)**
Not relevant	Not relevant	**Positive**	**Contamination Repeat experiment**

Tm values shift depending on the instrument, heating speed, mastermix/ salt contents and detection format. Single peak with lower Tm values are an indication for the presence of another mutation; missing peaks could be due to a drop out of the primer binding site. Temperatures with 1step RT pol. 90-9999-96 are 3-4°C higher.

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

Mutations in SARS-CoV-2 spike protein and RNA pol. are associated with COVID-19 mortality risk. Hahn et al., 2020

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

V201222	Release version	2020-12-22
V210122	10. Tm values for 1-step RT polymerase 90-9999-96	2021-01-22
V210123	8.2.3 Instructions for 1-step RT polymerase 90-9999-96	2021-02-04
V210303	Forward primer moved to include codon 484 (as used in 53-0789)	2021-03-03
V210404	Reverse primer moved (as used in 53-0797)	2021-04-04
V211111	Pos. control changed to ivRNA N501	2021-11-11

Certificate of Analysis (CoA)

Lot n° **5057**

Expiry : **YYYY-MM-DD**

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	501N	501Y		PC	passed
Tm range	55-58°C	60-63°C	**Cp range**	-	
Measured				-	✓
Signal level	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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