TIB MOLBIOL

Instructions For Use

VirSNiP SARS-CoV-2 Spike 371L 373P 452R 530

Cat.-No. 53-0831-96 Roche SAP n° 09 651 128 001

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

1. Content, Storage and Expiry

1 Vial yellow cap 96 reactions SARS CoV (lyophilized)

Storage at Arrival:

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.

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

Hundreds of thousands of isolates have been sequenced since the SARS-CoV-2 genome was published (MN908947). The novel **Omicron** variant is derived from Alpha, but carrying many new mutations.

Spike Prot. Variation	Genetic Variation	Alpha B.1.1.7	Beta B.1.351	Gamma B.1.1.28	Delta B.1.617.2	Omicron B.A1	Function, Effect	Assay
del HV69/70	del21765-770	x				x	evasion of immune response	53-0781
ins214EPE						x		53-0826
S371L S373P	T22673C C22674T					x		53-0827
K417T	A22812C			x			RBD (ACE binding domain)	53-0817
K417N	G22813T		x			x	RBD (ACE binding domain)	53-0787
L452R	T22917G				x		ACE binding, immune escape	53-0788
E484K	G23012A		x	x		A	RBD (ACE binding domain)	53-0789
N501Y	A23063T	x	x	x		+505	RBD (stronger ACE binding)	53-0780
P681H/R	C23604A/G	H			R	H	furin cleavage site	53-0786

4. Description

A 111 bp long fragment is amplified and analyzed in a melting curve, using a 452R specific probe. A 108 bp bp PCR long fragment is amplified and analyzed with a 371L and 373P specific probe.

5. Specification

Sensitivity not tested.

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 a 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

- not provided -

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

530: Amplification	530: Melting Curve	530: Melting Peak

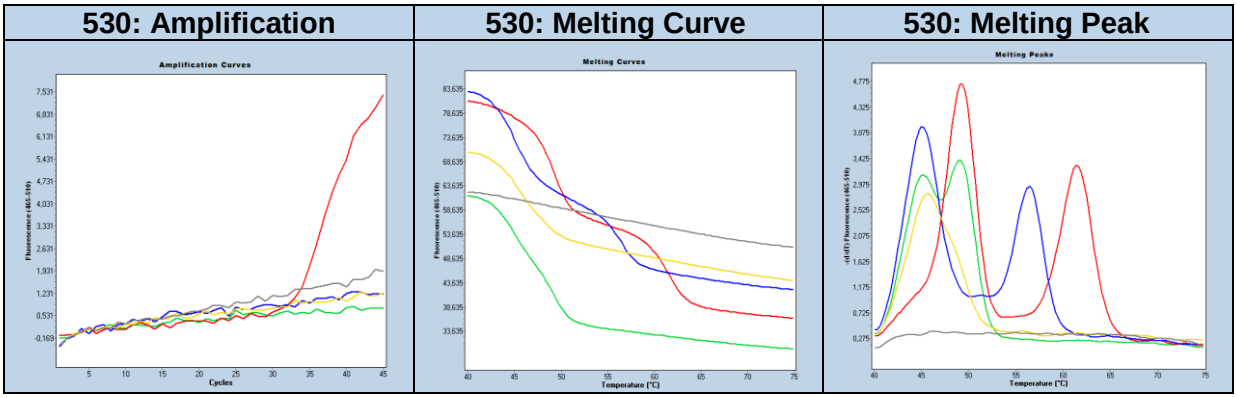

Figure 1: Red Omicron (BA.1), blue Delta, yellow Lambda, green wild type, grey NTC. **Left:** Only Omicron visible. **Center** Melting curves. **Right** L452 has a Tm of 49 (± 2)°C, 452R a Tm 56 (± 2)°C 371L/373PTm 61.5 (± 2)°C

10. Reading the Results

Use the Second Derivative Maximum method (Automated (F'' max). View results in the 530 channel. The negative control (NTC) must show no signal. For the melting curve analysis use 'Tm calling'.

Channel 530 Melting Temperature (Tm) °C				Result	Reference Strain (example)
~45°C*	~61.5°C*	~49°C*	~56°C*		
X		X		S371 S373 + L452	Not Delta not Omicron
X			X	S371 S373 + 454K	SARS Delta (or Kappa)
X				S371 S373 + 453R	SARS mink type
X		shoulder		S371 S373 + 454Q	SARS Lambda
	X	X		371L 373P + L452	SARS Omicron BA.1
	~55°C**	X		S371 373P + L452	SARS Omicron BA.2

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 ** expected Tm value
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 isolates reported as VOI and VOC.

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/en/publications-data/threat-assessment-brief-rapid-increase-sars-cov-2-variant-united-kingdom
Circulating SARS-CoV-2 spike var. N439K maintains fitness while evading antibody-mediated immunity. Thomson et al., 2020
Mutations in SARS-CoV-2 spike protein and RNA pol. are associated with COVID-19 mortality risk. Hahn et al., 2020
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

V211210 Release version 2021-12-10

Certificate of Analysis (CoA)

Lot n° **5169**
Expiry : **YYYY-MM-DD**

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	S371 S373	371L 373P	L452	452R		PC	passed
Tm range	44-46°C	60-63°C	48-50°C	55-58°C	**Cp range**	-	
Measured						-	✓
Signal level	2-10	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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