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 Mixed positive control 501N/Y, wt/69,70del, 681P/H

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

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 is reported to be more contagious. Spike protein variants del69/70, del144, **N501Y**, A570D, P681H, T716I, S982A and D1118H are marker for this cluster.

Spike Prot. Variation	Genetic Variation	UK* B.1.1.7	ZA 501.V2	Nigeria	DK mink V	Function, Effect	Assay
del HV69/70	del21765-770	**x**			x	evasion of immune response	53-0781
K417N	G22813T		x			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			RBD (ACE binding domain)	53-0789
N501Y	A23063T	**x**	x			RBD (stronger ACE binding)	53-0780
D614G	A23403G	**x**	x	x	x	RBD (stronger ACE binding)	53-0782
P681H	C23604A	**x**		x		furin cleavage site	53-0786
V1176F	G25088T					increased mortality	53-0784

4. Description

A 130 bp long fragment is amplified and analyzed running a 501Y-specific probe-based melting curve. 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. For other instruments use the SYBR Green melting option.

8.1. Programming Roche 480 Instruments

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	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

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 an **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 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:

For use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
10.4 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.4 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
0.1 µl	**RT Enzyme** (see Roche manual)	0.1 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 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

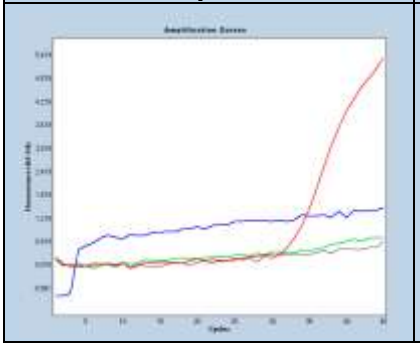
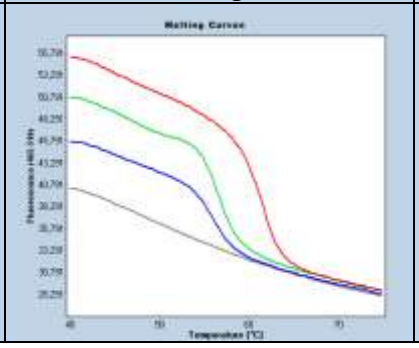
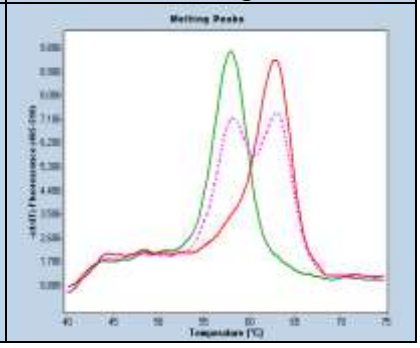
530: Amplification	530: Melting Curve	530: Melting Peak
		

Figure 1. 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

We recommend using 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 Amplification	Channel 530 Melting analysis	Channel 530 NTC Control	Result
Not relevant	Not relevant	**Negative / no peak**	**No virus amplified / not detectable**
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**

11. References

Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a 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

The circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity. Thomson et al., 2020

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

12. Multiplex PCR Compatibility Respiratory Virus Panel

- no tested -

13. Version History

V201222	Release version	2020-12-22
V201227	Mutation table, references added, DOM	2020-12-27
V210101	1. Content / 8.2.2 Positive control included, 5. Sensitivity range	2020-12-31

Certificate of Analysis (CoA)

Lot n° **4994**
Expiry : **YYYY-MM-DD**

	501N	501Y	wtRNA		PC	passed
Tm range	55-57°C	60-62°C	55-57°C	**Cp range**	27-30	
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*

TIB MOLBIOL Syntheselabor GmbH | Eresburgstr. 22-23 | D-12103 Berlin | Germany
Tel. +49 30 78 79 94 55 | FAX +49 78 79 94 99 | dna@tib-molbiol.de | WWW.TIB-MOLBIOL.COM
Geschäftsführer (CEO): Olfert Landt | Register HRB 93163 B | Registergericht Berlin Charlottenburg

Instructions For Use

VirSNiP SARS-CoV-2 Spike del H69/V70 530

Cat.-No. 53-0781-96 Roche SAP n° 09 405 674 001

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 CoV (lyophilized)
1 Mixed positive control 501N/Y, wt/69,70del, 681P/H

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 multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex RNA Virus Master Cat.-No. 06 754 155 001

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 is reported to be more contagious. Spike protein variants **del69/70**, del144, N501Y, A570D, P681H, T716I, S982A and D1118H are marker for this cluster.

Spike Prot. Variation	Genetic Variation	UK* B.1.1.7	ZA 501.V2	Nigeria	DK mink V	Function, Effect	Assay
del HV69/70	del21765-770	**x**			x	evasion of immune response	53-0781
K417N	G22813T		x			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			RBD (ACE binding domain)	53-0789
N501Y	A23063T	**x**	x			RBD (stronger ACE binding)	53-0780
D614G	A23403G	**x**	x	x	x	RBD (stronger ACE binding)	53-0782
P681H	C23604A	**x**		x		furin cleavage site	53-0786
V1176F	G25088T					increased mortality	53-0784

4. Description

A 119 (113) bp long fragment is amplified and analyzed running a melting curve using a deletion-specific detection probe. The amplification of isolates containing the wild type variant is barely 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 the upper part. Typical clinical samples are throat or 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. For other instruments use the SYBR Green melting option.

8.1. Programming Roche 480 Instruments

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	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

Table 1

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

Detection Format Multi Color HybProbe Detection Formats
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 an **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 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:

For use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
10.4 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.4 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
0.1 µl	**RT Enzyme** (see Roche manual)	0.1 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 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

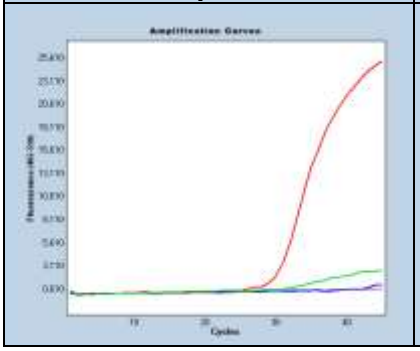
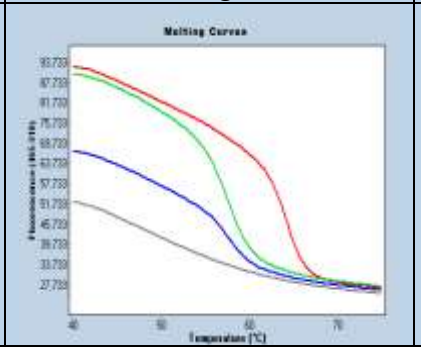
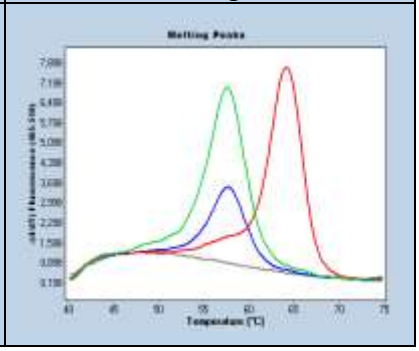
530: Amplification	Melting Curve	Melting Peak
		

Figure 1. Blue SARS RNA, Green wt, Red deletion pos control **Left** Deletion is clearly visible in the amplification. **Center**: Melting curves. **Right** Wt has a melting temperature of 57.8 (± 1)°C, deletion has a Tm of 64.0 (± 1)°C.

10. Reading the Results

We recommend using 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 Amplification	Channel 530 Melting analysis	Channel 530 NTC Control	Result
Not relevant	Not relevant	**Negative / no peak**	**No virus amplified / not detectable**
Invisible or low	**Tm ~ 57.8°C**	**Negative**	**SARS Spike wild type (not UK variant)**
Visible	**Tm ~ 64.0°C**	**Negative**	**SARS Spike del69/70 (UK or cluster V)**
Not relevant	Not relevant	**Positive**	**Contamination Repeat experiment**

11. References

Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a 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

The circulating SARS-CoV-2 spike variant N439K maintains fitness while evading antibody-mediated immunity. Thomson et al., 2020

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

12. Multiplex PCR Compatibility Respiratory Virus Panel

- no tested -

13. Version History

V201227	Release version	2020-12-22
V210101	1. Content / 8.2.2 Positive control included, 5. Sensitivity range	2021-01-06

Certificate of Analysis (CoA)

Lot n° **4996**
Expiry : **YYYY-MM-DD**

	wt	del69/70	wtRNA		PC	passed
Tm range	57-58°C	63-65°C	57-58°C	**Cp range**	27-30	
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*

TIB MOLBIOL Syntheselabor GmbH | Eresburgstr. 22-23 | D-12103 Berlin | Germany
Tel. +49 30 78 79 94 55 | FAX +49 78 79 94 99 | dna@tib-molbiol.de | WWW.TIB-MOLBIOL.COM
Geschäftsführer (CEO): Olfert Landt | Register HRB 93163 B | Registergericht Berlin Charlottenburg

TIB MOLBIOL

Instructions For Use

VirSNiP SARS-CoV-2 Spike E484K

530

Cat.-No. 53-0789-96

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)
1 Mixed positive control

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.
- 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

The SARS-CoV-2 genome was published 11.1.20 (Genbank MN908947). Hundreds of thousand isolates have been sequenced. The UK and the South Africa variant are reported to be more contagious. Spike protein variants 417N, **484K**, 501Y and 614G are marker for the South African cluster.

Spike Prot. Variation	Genetic Variation	UK* B.1.1.7	ZA B.1.351	Nigeria B.1.1.238	Brasil B.1.1.28	DK mink Clust V	Function, Effect	Assay
del HV69/70	del21765-770	**x**				x	evasion of immune response	53-0781
K417N	G22813T		x				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		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	RBD (stronger ACE binding)	53-0782
P681H	C23604A	**x**		x			furin cleavage site	53-0786
V1176F	G25088T				x		increased mortality	53-0784

4. Description

A 76 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 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 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:

For use with the Roche LightCycler® Multiplex RNA Virus Master		
for 5 µl extract	**Component**	**10 µl extract**
10.4 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.4 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
0.1 µl	**RT Enzyme** (see Roche manual)	0.1 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 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

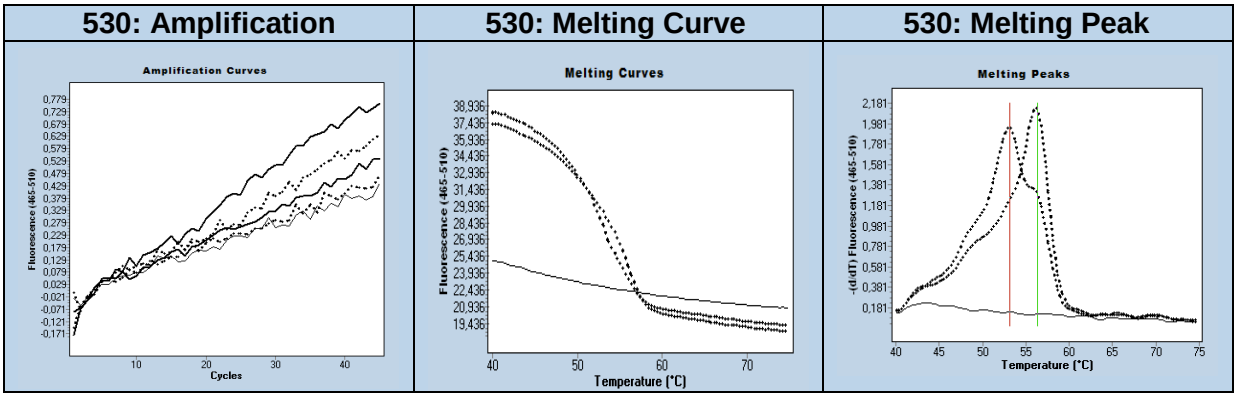


Figure 1. **Left** Amplification not visible. **Center** Melting curves. **Right** 484E has a melting point of 53°C (± 2)°C, 484K has a Tm of 56°C (± 2)°C. Positive control is a mixture 484Q and 484K

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 Amplification	Channel 530 Melting analysis	Channel 530 NTC Control	Result
Not relevant	Not relevant	**Negative / no peak**	**No virus amplified / not detectable**
Invisible	**Tm ~ 51.0°C***	**Negative**	**SARS Spike 484Q (not ZA variant)**
Invisible	**Tm ~ 53.1°C***	**Negative**	**SARS Spike 484E (not ZA variant)**
Invisible	**Tm ~ 56.3°C***	**Negative**	**SARS Spike 484K (poss. 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 Positive control is a mixture 484Q and 484K

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

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 10. Tm values for 1-step RT polymerase 90-9999-96	2021-01-22

Certificate of Analysis (CoA)

Lot n° **5005**

Expiry : **YYYY-MM-DD**

TIB MOLBIOL

	484E	484K	484Q		PC	passed
Tm range	53-55°C	56-59°C	48-51°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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