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MutaPLEX® CoV-2 MUT

Real-Time-RT-PCR Kit

For the simultaneous differentiation of the novel coronavirus (SARS-CoV-2), lineages B.1.1.7 (UK), B.1.351 (ZA) and P.1 (BRA)

Valid from 2021-03-08

REF **KG193196**

Σ 96

-20 °C

IVD

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Table of Contents

1 INTENDED USE

The MutaPLEX® CoV-2 MUT Real-Time RT-PCR Kit is an assay for the simultaneous differentiation of the novel coronavirus (SARS-CoV-2) lineages B.1.1.7 (United Kingdom, UK), B.1.351 (South Africa, ZA) and P.1 (Brasilia, BRA) from biological specimens.

2 PRINCIPLE OF THE TEST

The MutaPLEX® CoV-2 MUT Real-Time RT-PCR Kit contains four specific primer and probe systems for the detection of SARS-CoV-2 spike protein mutations. Each mutation is related to one specific dye. If one of the mutations is present, the specific channel will show no or a delayed signal. The mutations covered with this assay are del69/70 (FAM), del 241-243 (ROX), N501Y (HEX) and H655Y (Cy5). In case of B.1.1.7 (UK), B.1.351 (ZA) or P.1 (BRA), the HEX channel and one additional channel, depending on the mutation, will show no or a delayed signal (Table 7). In this case, the other two channels should show a normal signal.

3 PACKAGE CONTENTS

The reagents supplied are sufficient for 96 reactions.

Table 1: Components of the MutaPLEX® CoV-2 MUT Real-Time-RT-PCR Kit .

Label	Lid Colour	Content
Reaction Mix	yellow	1 x 1 325 µl
Dilution Buffer	white	2 x 960 µl
Enzyme	blue	1 x 19.2 µl
Positive Control	red	1 x 150 µl

4 EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- Sterile microtubes
- Calibrated precision pipets (adjustable volume) and sterile single-use tipps with filter
- Disposable gloves
- Table centrifuge
- Vortex
- Real-Time PCR instrument
- Optical PCR reaction tubes with lid or optical PCR reaction plate with optical foil
- Optional: Liquid handling system for automation

5 TRANSPORT, STORAGE AND STABILITY

The MutaPLEX® CoV-2 MUT Real-Time RT-PCR Kit is shipped on dry ice or cool packs. All components must be stored at maximum -20 °C in the dark immediately after receipt. Do not use reagents after the date of expiry printed on the package. Up to 20 freeze and thaw cycles are possible. For convenience, opened reagents can be stored at +2 to +8 °C for up to 6 months. Protect kit components from direct sunlight during the complete test run.

6 WARNINGS AND PRECAUTIONS

Read the Instruction for Use carefully before using the product.

Before first use check the product and its components for:

- Use of this product is limited to personnel specially instructed and trained in the techniques of Real-Time PCR procedures.
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Avoid microbial and nuclease (DNase/RNase) contamination of the eluates and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (1) sample preparation, (2) reaction setup and (3) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organisations.

- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

7 SAMPLE MATERIAL

Starting material for MutaPLEX® CoV-2 MUT Real-Time RT-PCR Kit is RNA qualified SARS-CoV-2 positive by Real-Time RT-PCR (e.g. MutaPLEX Coronavirus Real-Time RT-PCR Kit, Imundiagnostik, Cat. No. KG 1926).

Eluates with very low copy numbers resulting in CT values >32 are not suitable for testing with the MutaPLEX® CoV-2 MUT Real-Time RT-PCR.

8 SAMPLE PREPARATION

The eluted RNA has to be diluted 1:10 in Dilution Buffer to optimize the condition for the mutation detection. **Do not use other buffers than the Dilution buffer included in the MutaPLEX® CoV-2 MUT Real-Time RT-PCR Kit.**

Table 2: Preparation of the Sample

Volume per Reaction
2 µl eluted RNA
18 µl Dilution Buffer

9 REAL-TIME-RT-PCR

9.1 Important points before starting

- Please pay attention to chapter 7 "Warnings and precautions".
- Before setting up the Real-Time-RT-PCR familiarise yourself with the Real-Time PCR instrument and read the user manual supplied with the instrument.
- The programming of the thermal profile should take place before the RT-PCR set up.
- In every RT-PCR run one Positive Control and one ‚no template control' (Dilution Buffer) should be included.

- Before each use, all reagents should be thawed completely at room temperature, thoroughly mixed (except the Enzyme) and centrifuged very briefly.
- Due to the high viscosity of the Enzyme (blue lid), prewarming at room temperature for 15 min is recommended.

9.2 Procedure

Prepare the Master Mix according to Table 3.

The master mix contains all of the components needed for RT-PCR except the sample. Prepare a volume of master mix for at least one sample more than required, in order to compensate for pipetting inaccuracy.

Table 3: Preparation of the master mix

Volume per reaction	Volume master mix
13.8 µl Reaction Mix	13.8 µl x (N+1)
0.2 µl Enzyme	0.2 µl x (N+1)

Real-Time-RT-PCR set up

- Place the number of optical PCR reaction tubes needed into the respective tray of the Real-Time PCR instrument / take an optical PCR reaction plate.
- Pipet 14 µl of the Master Mix into each optical PCR reaction tube / the optical PCR reaction plate.
- Add **6 µl** of the diluted eluates, the Positive Control and the ‚no template control' to the corresponding optical PCR reaction tube / the optical PCR reaction plate (Table 4).
- Close the optical PCR reaction tubes / the optical PCR reaction plate immediately after filling in order to reduce the risk of contamination.

Table 4: Preparation of the Real-Time-RT-PCR

Component	Volume
Master mix	14.0 µl
Sample	6.0 µl
Total volume	20.0 µl

9.3 Instrument settings

For the Real-Time-RT-PCR use the thermal profile shown in table 5.

Table 5: Real-Time-RT-PCR thermal profile

Description	Time	Temperature	Number of Cycles	Aquisitions
Reverse Transcription	10 min	45 °C	1	no
Initial Denaturation	5 min	95 °C	1	no
Denaturation	10 sec	95 °C	45	no
Annealing and Extension	40 sec	60 °C		end of step

Dependent on the Real-Time instrument used, further instrument settings have to be adjusted according to table 6.

Table 6: Overview of the instrument settings required for the MutaPLEX® CoV-2 MUT Real-Time-RT-PCR.

Real-Time-PCR-Instrument	Parameter Reaction Mix	Detection channel	Notes
LightCycler 480II	del69/70 (UK) N501Y (UK, ZA, BRA) del241-243 (ZA) H655Y (BRA)	465–510 533–580 533–610 618–660	Colour Compensation Kit MutaPlex® CC-1 (KG19-5-CC) is required Melt factor: 1 / Quant factor: 10 / Max integration time (s): 1 Melt factor: 1 / Quant factor: 10 / Max integration time (s): 2 Melt factor: 1 / Quant factor: 10 / Max integration time (s): 2 Melt factor: 1 / Quant factor: 10 / Max integration time (s): 3
QuantStudio 5 Bio-Rad CFX96	del69/70 (UK) N501Y (UK, ZA, BRA) del241-243 (ZA) H655Y (BRA)	FAM HEX ROX Cy5	Reference Dye: None
NEOS-48 qPCR NEOS-96 qPCR	del69/70 (UK) N501Y (UK, ZA, BRA) del241-243 (ZA) H655Y (BRA)	FAM HEX ROX Cy5	Reference Dye: None

Real-Time-PCR-Instrument	Parameter Reaction Mix	Detection channel	Notes
Mic qPCR Cycler	del69/70 (UK) N501Y (UK, ZA, BRA) del241-243 (ZA) H655Y (BRA)	Green Yellow Orange Red	Gain 8 Gain 10 Gain 10 Gain 10

9.4 Thresholds

To ensure the quality of the PCR, it is advised to set the thresholds (except LC480II) based on the positive control provided with the kit. The Ct values for the FAM, ROX and Cy5 channel should be at 19 – 22. The Ct for HEX should be 22 – 25, app. 3 - 4 Ct after the FAM signal.

10 DATA ANALYSIS

10.1 Interpretation of the PCR Signals

Positive Signal: There are up to 4 curves present for each sample. For Wildtype samples (Wuhan like SARS-CoV-2) the C_T values for all dyes of those curves should cluster within a range of 6 C_T. All signals in the range of +6 C_T after the earliest signal, should be accounted as positive signal (examples in Fig.1 - 6).

Negative Signal: In case of a mutation, different fluorescence channels will show no amplification. For higher concentrated samples, a decreased signal might appear in the FAM, HEX, ROX or Cy5 channel. In this case, the C_T values have to be aligned in relation to the earliest C_T. If the decreased signal shows a difference of 6 C_T or more, the Signal should be accounted as negative signal.

Cutoff: There is no explicit cutoff for the PCR, but if there are results with a single curve for only one dye, showing a Ct of > 40, it is recommended to repeat the PCR with a new dilution, as suggested in the 'trouble shooting' section for very weak samples.

Figure 1, Figure 2, Figure 3, Figure 4, Figure 5 and **Figure 6** show examples for Real-Time RT-PCR results of the wildtype and different lineages of SARS-CoV-2.

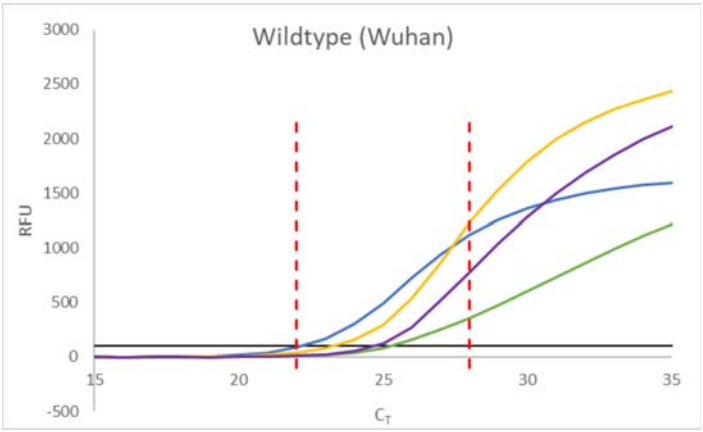

Fig 1: Detection of Wuhan like SARS-CoV-2 RNA. All four channels (blue: FAM, green: HEX: orange: ROX, purple: Cy5) show a positive signal and the CT values are close to each other (less than 6 CT between the first and the last signal, red lines).

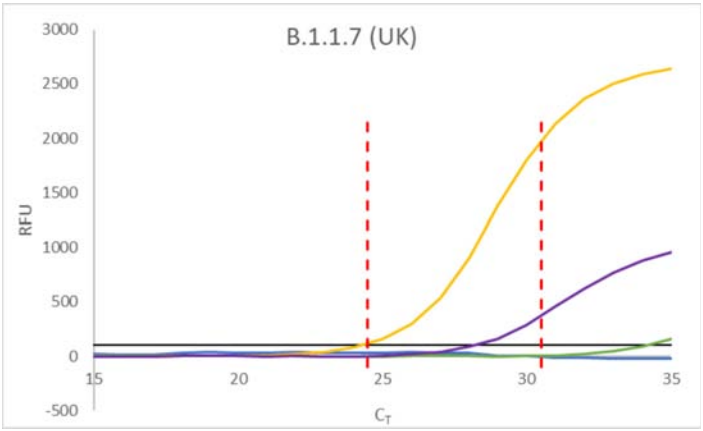

Fig 2: Detection of Lineage B.1.1.7 (UK) SARS-CoV-2 RNA. Only the signals of two channels (orange: ROX, purple: Cy5) count as positive. The FAM channel (blue) shows no signal. The HEX channel (green) is out of range (red lines).

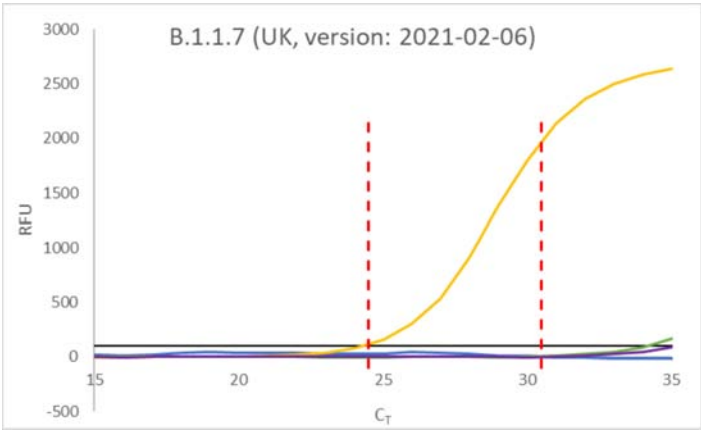

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Fig 3: Detection of Lineage B.1.1.7 (UK) SARS-CoV-2 RNA. Only the signals of one channel (orange: ROX) count as positive. The FAM channel (blue) shows no signal. The HEX channel (green) and the Cy5 channel (purple) are out of range (red lines).

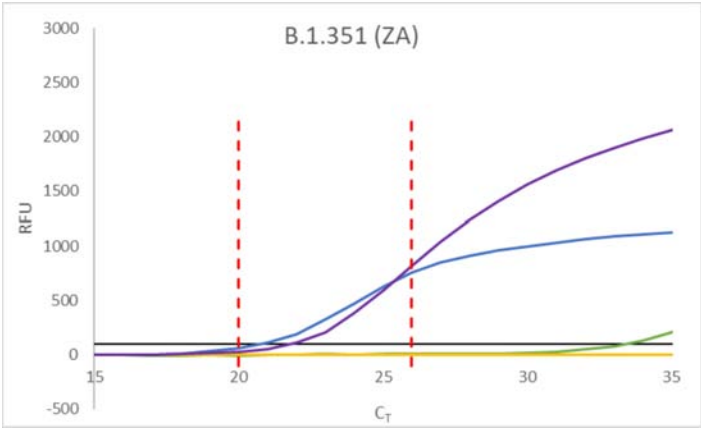

Fig 4: Detection of Lineage B.1.351 (ZA) SARS-CoV-2 RNA Only the signals of two channels (blue: FAM, purple: Cy5) count as positive. The ROX channel (orange) shows no signal. The HEX channel (green) is out of range (red lines).

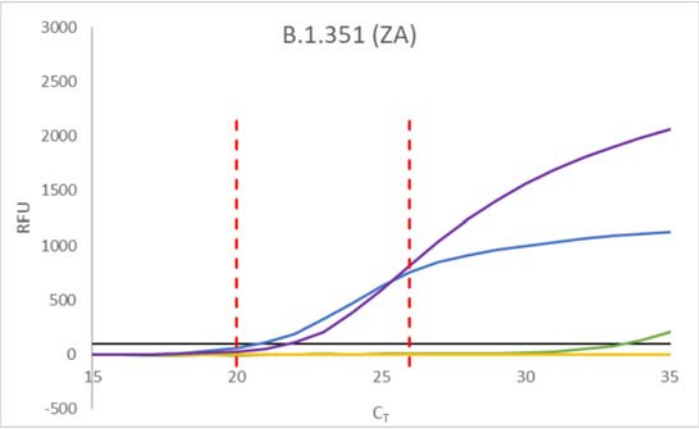

Fig 5: Detection of Lineage P.1 (BRA) SARS-CoV-2 RNA Only the signals of two channels (blue: FAM, orange: ROX) count as positive. The HEX channel (green) and the Cy5 channel (purple) are out of range (red lines).

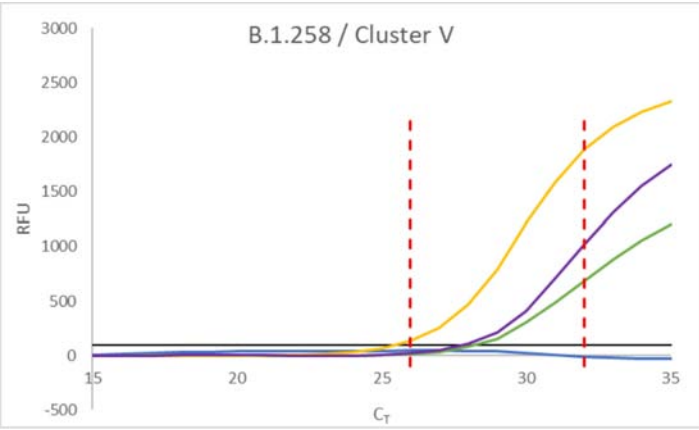

Fig 6: Detection of Lineage B.1.258 SARS-CoV-2 RNA. The signals of three channels (orange: ROX, green: HEX, purple: Cy5) count as positive. The FAM channel (blue) shows no signal.

10.2 Interpretation of the Results

Table 7: Interpretation of the results for MutaPLEX® CoV-2 MUT

Signal/ C_T-Value				Interpretation
FAM (UK)	**HEX**	**ROX (ZA)**	**Cy5 (BRA)**	
del69/70	N501Y	del241-243	H655Y	
positive	**positive**	**positive**	**positive**	**Positive result, the sample contains SARS-CoV-2 Wildtype RNA. No Variant of Concern.**
negative / out of range	negative / out of range	**positive**	**positive or negative / out of range**	**Positive result, the sample contains SARS-CoV-2-RNA Lineage B.1.1.7 (UK).**
positive	negative / out of range	negative / out of range	**positive**	**Positive result, the sample contains SARS-CoV-2-RNA Lineage B.1.351 (ZA).**
positive	negative / out of range	**positive**	negative / out of range	**Positive result, the sample contains SARS-CoV-2-RNA Lineage P.1 (BRA) or A.27 (France)[1].**
negative / out of range	**positive**	**positive**	**positive or negative / out of range**	**Positive result, the sample contains SARS-CoV-2-RNA Lineage B.1.258. No Variant of Concern.[2]**
positive	negative / out of range	**positive**	**positiv**	**Positive result, the sample contains SARS-CoV-2 RNA carrying the Spike: N501Y mutation. No Variant of Concern.**
positive	**positive**	**positive**	negative / out of range	**Positive result, the sample contains SARS-CoV-2 RNA carrying the Spike: H655Y mutation. No Variant of Concern.**
negative	negative	negative	negative	**Negative result. The sample must be repeated.**

[1] P.1 (Variant of Concern) and A.27 can not be distinguished by the PCR. The sample needs confirmation by another method.

[2] The mutation pattern implies SARS-CoV-2 Cluster V (mink, DK) as well, but this variant is officially eradicated.

11 ASSAY VALIDATION

No template Control

The ‚no template control' must show no C_T in the FAM, HEX, ROX and Cy5 channel.

Positive controls

All parameters in the Positive Control must show a positive (i.e. exponential) amplification curve in the different channels FAM, Cy5, ROX and HEX. The Positive Control must fall below a C_T of 30.

12 LIMITATIONS OF THE METHOD

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of Real-Time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay.
- All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- This assay must not be used on a biological specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- As with any diagnostic test, results of the MutaPLEX® CoV-2 MUT Real-Time-RT-PCR Kit need to be interpreted in consideration of all clinical and laboratory findings.

13 TROUBLESHOOTING

The following troubleshooting guide is included to help you with possible problems that may arise when performing a Real-Time RT-PCR. If you have further questions, please do not hesitate to contact our scientists on info@immundiagnostik.com.

No fluorescence signal in the FAM and/or ROX and/or Cy5 and/or HEX of the positive control

The selected channel for analysis does not comply with the protocol

Select the detection channels according to table 6.

Incorrect preparation of the Master Mix

Make sure the enzyme is added to the master mix (chapter 9).

Incorrect configuration of the Real-Time-RT-PCR

Check your work steps and compare with chapter 9.

The programming of the thermal profile is incorrect

Compare the thermal profile with the protocol 'Instrument Settings' in Table 5 and Table 6.

Incorrect storage conditions for one or more kit components or kit expired

Check the storage conditions and the date of expiry printed on the kit label. If necessary, use a new kit and make sure kit components are stored as described in chapter Transport, storage and stability.

Detection of a fluorescence signal in the FAM and/or ROX and/or Cy5 and/or HEX channel of the ‚no template control'.

Contamination during preparation of the Real-Time RT-PCR

Repeat the Real-Time RT-PCR in replicates. If the result is negative in the repetition, the contamination occurred when the samples were pipetted into the optical PCR reaction tubes. Make sure to pipet the Positive Control last and close the optical PCR reaction tube immediately after adding the sample. If the same result occurs, one or more of the kit components might be contaminated. Make sure that workspace and instruments are decontaminated regularly. Use a new kit and repeat the Real-Time RT-PCR.

No or very weak signals for weak, prequalified samples with Ct 32 in the screening PCR.

Sensitivity and Fluorescence intensity may differ on individual Real-Time PCR Cycler.

Retest the sample in a new dilution, using 4 µl sample and 18 µl dilution buffer.

14 KIT PERFORMANCE

The adjustment and validation of the MutaPLEX® CoV-2 MUT Real-Time RT-PCR kit is an ongoing process. Hence, comparison data from sequences of eluted SARS-CoV-2 RNA are evaluated continuously.

Detailed information based on the latest state of knowledge is available at Immundiagnostik AG. Please address your inquiry to info@immundiagnostik.com.

15 ABBREVIATIONS AND SYMBOLS

RT-PCR	Reverse transcription-PCR	REF	Catalog number
RNA	Ribonucleid acid	IVD	*In vitro* diagnostic medical device
REACTION MIX	Reaction mix	Σ	Contains sufficient for <n> test
ENZYME	Enzyme		Upper limit of temperature
CONTROL +	Positive control		Manufacturer
DILUTION BUFFER	Dilution Buffer		Use by YYYY-MM-DD
LOT	Lot number		Consult instructions for use
→REF	use with	CONT	Content

16 LITERATURE

1. www.who.int/health-topics/coronavirus
2. Rambaut et al. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations. nCoV-2019 Genomic Epidemiology
3. Garry. Mutations arising in SARS-CoV-2 spike on sustained human-to-human transmission and human-to-animal passage. nCoV-2019 Genomic
4. Faria et al. Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings. nCoV-2019 Genomic Epidemiology