# *UltraGene Assay*

## *SARS-CoV-2 VOC Screening & Determination*

## V1.X

**50**
**100**
**1000**

# User Guide

Version 1 – Revision 1

Qualitative SARS-CoV-2 assay - For use with qPCR Instruments

**For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.**

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## Application

**For Research Use Only (RUO).** Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) test (nucleic acid technique (NAT)) intended to screen the emergence of SARS-CoV-2 genome harboring the mutations L452R, K417N/T, E484A/K and the deletion 69-70 on the Spike (S) gene. The test is targeting the S and N regions of SARS-CoV-2 patients' extracted RNA and already diagnosed PCR positive to SARS-CoV-2.

***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR

## Principles of the assay

The ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) is a real-time reverse transcription polymerase chain reaction (rRT-PCR) multiplex test which includes five primer and probe sets designed to detect RNA from the SARS-CoV-2 Spike (S) and N genes in extracted RNA from individuals already tested SARS-CoV-2 positive. Each set is available in a distinct well.

As the specimen is already tested positive, the N gene is used as a reverse transcription and PCR amplification positive control for each well. RNA priorly isolated from upper respiratory specimens (e.g. nasopharyngeal swab samples) is reverse transcribed to cDNA and subsequently amplified using the real-time PCR instrument. Melt curve profile is different depending on the targeted mutation. Deletion 69-70 when present doesn't have a Ct value, "S-gene dropout".

During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase and molecular beacon probe degrade the bound probe, causing the reporter dyes (FAM, HEX, ROX and Cy5) to separate from the quencher dye (BHQ2), generating fluorescent signals (amplification curve or melt curve). Fluorescence intensity is monitored at each PCR cycle.

Note: the preliminary analytical performances were established using nasopharyngeal swab samples. oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with the Assay

## Assay components

The ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) is provided in 3 formats of 50, 100 and 1000 reactions, REF 176A50, REF 176A100 and REF 176A1000, respectively.

| Label | Reagent | Description |
|---|---|---|
| **Master Mix** | RT-PCR Premix | The master mix consists of a MgSO4, dNTPs, and stabilizers (including buffer) |
| **Enzyme Mix** | Enzyme Premix | The enzyme mix is a version of Moloney Murine Leukemia Virus Reverse Transcriptase (M–MLV RT) that has been engineered to reduce RNase H activity and provide increased thermal stability. The enzyme DNA Polymerase is recombinant Taq DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling, providing an automatic "hot start" in PCR for increased sensitivity, specificity, and yield |
| **MgSO4** | Magnesium Sulfate | 50-mM Magnesium Sulfate to enhance the PCR |
| **Well #1 Primer & probe mix** | Primer/Probe Mix | S specific primer and probe set for mutation L452R, K417N/T and for Deletion Del 69-70; N specific primer and probe set for the internal control. |
| **Well #2 Primer & probe mix** | Primer/Probe Mix | S specific primer and probe set for mutation E484A/K; N specific primer and probe set for the internal control. |
| **$H_20$** | $H_20$ | PCR grade nuclease-free water |
| **Positive Control** | Positive Control | Positive control RNA |
| **Negative Control** | Negative Control | PCR grade nuclease-free water |

*Table 1: Components Included with the UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*

Storage of ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X***

| Label | Color Cap | Storage |
|---|---|---|
| **Master Mix** | Green | -25°C to - 15 °C |
| **Enzyme Mix** | Pink | -25°C to - 15 °C |
| **MgSO4** | Brown | -25°C to - 15 °C |
| **Well #1 Primer & probe mix** | Yellow | -25°C to - 15 °C |
| **Well #2 Primer & probe mix** | Orange | -25°C to - 15 °C |
| **$H_20$** | Blue | -25°C to - 15 °C |
| **Positive Control** | Clear | -25°C to - 15 °C |
| **Negative Control** | Black | Can be stored at -25°C to - 15 °C with other reagents |

*Table 2: Storage conditions of the UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*

## Reagent storage and handling

The ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) kit should be stored at - 25°C to - 15 °C and is stable until the expiration date stated on the label. Note the production date and expiration date listed on the label. Reagents from different lot numbers should not be mixed.

Note: Multiple thaw-freeze cycles should be avoided. Aliquoting should be considered.

## Materials required but not provided

- Any laboratory validated instrument for RNA extraction and purification using magnetic-bead technology.
- Any laboratory validated manual qPCR instrument (at least with FAM channel or equivalent).
- To be used for master mix preparation:
  - Eppendorf Safe-Lock Tubes 1,5mL Catalog #0030123328
  - Microliter pipets dedicated to PCR (0.1-2.5 µL; 1-10 or 1-20 µL; 20-200 µL; 1000 µL)
  - Adjustable pipettes & fitting filtered pipette tips
  - Benchtop centrifuge with rotor for 0.5 mL/1.5 mL reaction tubes (capable of attaining 10,000 rpm).
  - Benchtop vortex mixer.
  - Appropriate PPE & workspaces for working with potentially infectious samples
  - Surface decontaminants such as DNAZap (Life Technologies), DNA Away (Thermo Fisher Scientific), RNAse Away (Thermo Fisher Scientific), 10% bleach
  - Nuclease-free dH2O

Note: Ensure that instruments have been checked and calibrated according to the manufacturer's recommendations. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

## Warnings and precautions

- **For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.**
- This product has been tested only for nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- Handle all specimens as of infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2: https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafetyguidelines.html.

- Laboratories maybe required to report all test results to the appropriate public health authorities.
- Store assay reagents as indicated on their individual labels.
- Do not mix reagents from different kit lots.
- Reagents must be stored and handled as specified in these instructions for use. Do not use reagents past expiration date.
- Work surfaces and pipettes should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNAZap™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- The Positive Control should be handled in an area separate from sample receiving, accessioning and processing areas to avoid contamination of the samples with amplifiable material.
- Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- Dispose of waste in compliance with the local, state, and federal regulations. Frequent cleaning of the wells of the PCR instrument plate is recommended to prevent contamination. To avoid contamination, use separated and segregated working areas: 1) Reagent preparation area – preparing the reagents for amplification, 2) Dilution of positive control material, 3) sample preparation area- isolation of the RNA/ DNA from sample and control, and 4) Amplification area- amplification and detection of nucleic acid target.
- Check whether the PCR reaction tubes are tightly closed before loading on the PCR instrument to prevent contamination of the instrument from leaking tubes.

## Workflow

1) Batches of clinical samples tested for SARS-CoV-2.
    - Negative and positive results are given to healthcare provider
    - PCR positive results are kept for second testing

2) Extracted RNA from positive results of previous PCR runs are used with the ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO):
    a) PCR reaction setup
    b) PCR run
    c) Analysis of the presence or not of the K417N/T, L452R, E484A/K and the deletion 69-70 on the Spike (S) gene

If the PCR preparation reactions of the ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X (***RUO) are not carried out immediately after the SARS-CoV-2 detection PCR run:

- Extracted nucleic acid should be stored at -70°C or lower
- It is recommended to make few aliquots of RNA extracts to prevent thawing and unthawing

Note: As poor RNA quality might result in failure to amplify the targets, performing a new RNA isolation from initial upper respiratory specimen detected positive in a previous PCR run could be mandatory.

Do not vortex specimens as this will fragment the RNA and lead to failure of the ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) tests.

## PCR reaction setup

Note: Plate set-up configuration can vary with the number of specimens and workday organization.

**Vortexing the mix of "Master Mix" and "Primer and Probe Mix" reagents (Well #1 and Well #2) with or without extracted RNA solution, could lead to failures. As specificied in steps "4." and "5.", please don't vortex.**

1. Thaw the following reagents on ice (protected from light): Master Mix (RT-PCR Master Mix), Primer and Probe Mixes (for S and N genes targets in Well #1 and Well #2) and controls (Positive and Negative).
2. Briefly centrifuge (2000 rpm, 10sec) the reagents to collect the contents.
3. Set up a premix solution based on the number of sample preps to be tested. The volume of the premix required for all sample prep(s) to be tested = (number of sample preps + 2 controls) * the total volume of premix reagents (as listed in the table below).

| Reagent | Volume/ Reaction | |
|---|---|---|
| | Well #1 | Well #2 |
| **Master Mix** | 6.25 µL | 6.25 µL |
| **Enzyme Mix** | 0.50 µL | 0.50 µL |
| **MgSO4** | 0.40 µL | 0.40 µL |
| **Well #1 Primer & probe mix** | 13.00 µL | - |
| **Well #2 Primer & probe mix** | - | 6.50 µL |
| **$H_20$** | - | 6.35 µL |
| **Total volume** | 20.00 µL | 20.00 µL |

*Table 3: Volumes and storage conditions of the UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*

4. Evenly aliquot 20 µL of the Premix reagents into each qPCR tube (one qPCR tube per sample to be tested + 2 for the controls). **DON'T VORTEX.**
5. Add 5 µL of extracted RNA solution to a single qPCR tube. **DON'T VORTEX.**

Note: Do not add more than one sample of extracted RNA into a single qPCR tube. Each qPCR tube should have a total volume of 25 µL. Then immediately close the tubes and transfer the reaction setup into a qPCR machine for the amplification.

## PCR cycling conditions

Set up the cycling program for the laboratory validated real-time PCR instrument.

We give here example with the following real-time PCR instrument:

- qPCR Mic (Magnetic Induction Cycler) 4 Channels (Bio Molecular Systems, software version 2.10.5)

Note: Ensure that instrument has been checked and calibrated according to the manufacturer's recommendations. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

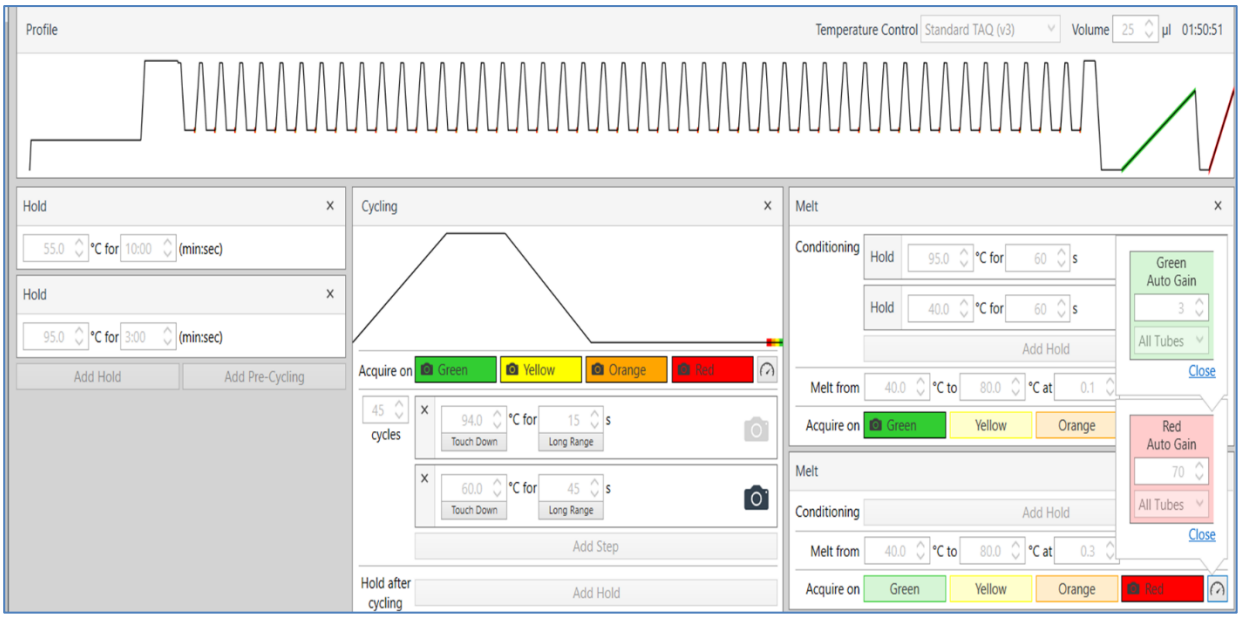



*Figure 1: PCR Cycling program for the qPCR Mic instrument*

## Selection of fluorescence channel

Please refer to the qPCR Mic instructions for use for the setup of the fluorescence channels FAM ( Green), HEX (Yellow), ROX (Orange) and Cy5 (Red) for the SARS-CoV-2 S and N targets.

# Results analysis per assay targets

## Mutations in K417 (Well #1)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **>= 69** | Cy5 | The sample is most likely positive for the K417N mutation | K417N |
| **66 – 68.5** | Cy5 | The sample is negative for the K417N mutation | K417K (wild type) |
| **< 66** | Cy5 | The sample is negative for the K417N mutation, but most likely contain a different mutation in the probe area | Other than K417N mutation (K417T) |
| **No peaks** | Cy5 | Not possible to determine genotype | Uninterpretable |

*Table 4: Analysis of melting peaks results for mutations in K417 with the qPCR Mic instrument (Well #1)*

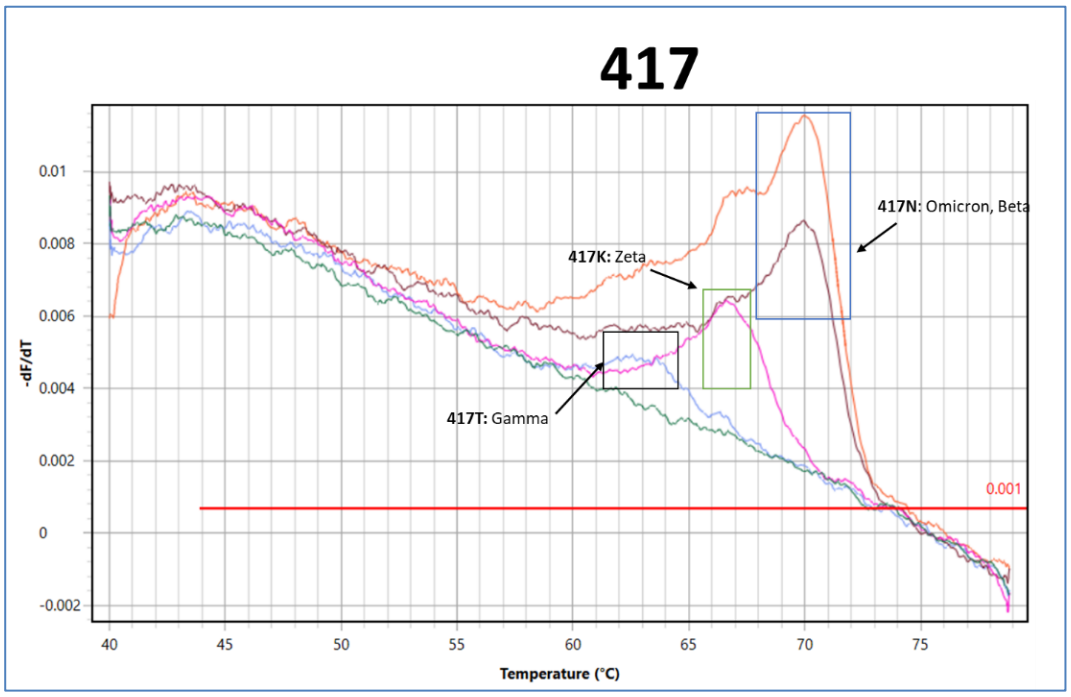



*Figure 2: Melting curves for the K417N target with qPCR Mic instrument (Well #1)*

## Mutations in L452 (Well #1)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **>= 66** | FAM | The sample is positive for the L452R mutation | L452R |
| **60 – 64** | FAM | The sample is negative for the L452R mutation | L452 Wild Type |
| **< 60** | FAM | The sample is negative for the L452R mutation, but most likely contain a different mutation in the probe area. | Other than L452R mutation |
| **No peaks** | FAM | Not possible to determine genotype | Uninterpretable |

*Table 5: Analysis of melting peaks results for mutations in L452 with qPCR Mic instrument (Well #1)*

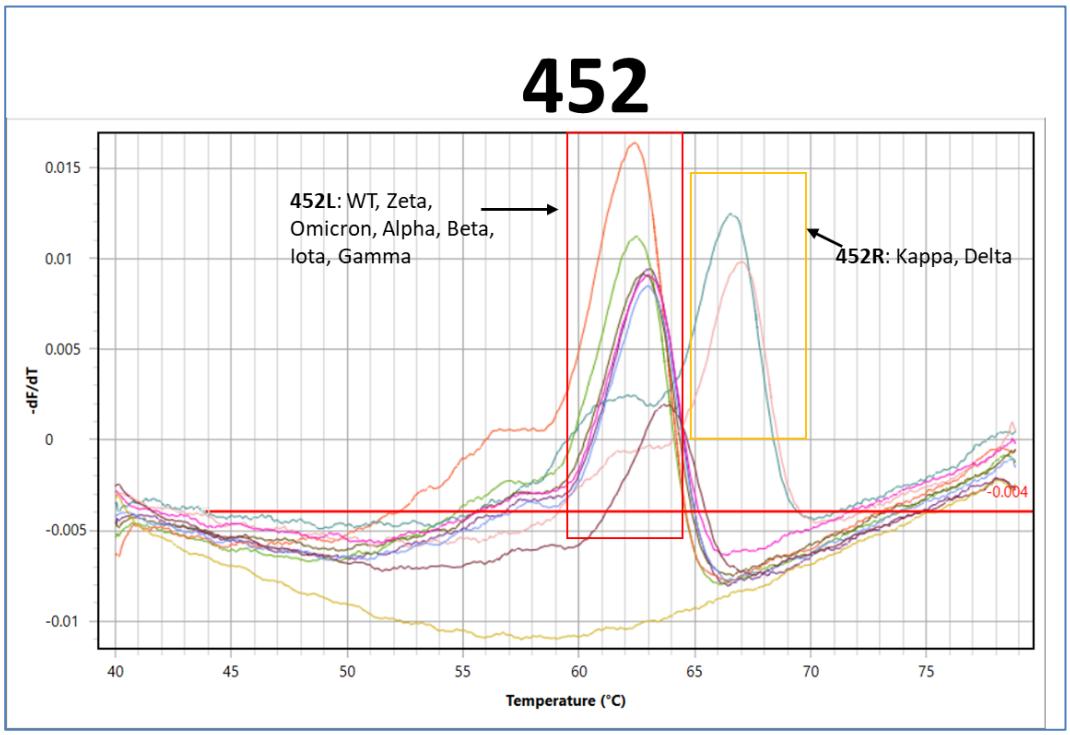



*Figure 3: Melting curves for the L452R target with qPCR Mic instrument (Well #1)*

## Deletion 69-70 (Well #1)

| Ct value | Channel | Analysis | Reporting |
|---|---|---|---|
| **N/A** | ROX | The sample is positive for the deletion | Presence of the deletion 69-70 |
| **> 10** | ROX | The sample is negative for the deletion | Absence of the deletion 69-70 |

*Table 6: Analysis of Ct value results for deletion 69-70 with the qPCR Mic instrument (Well #1)*

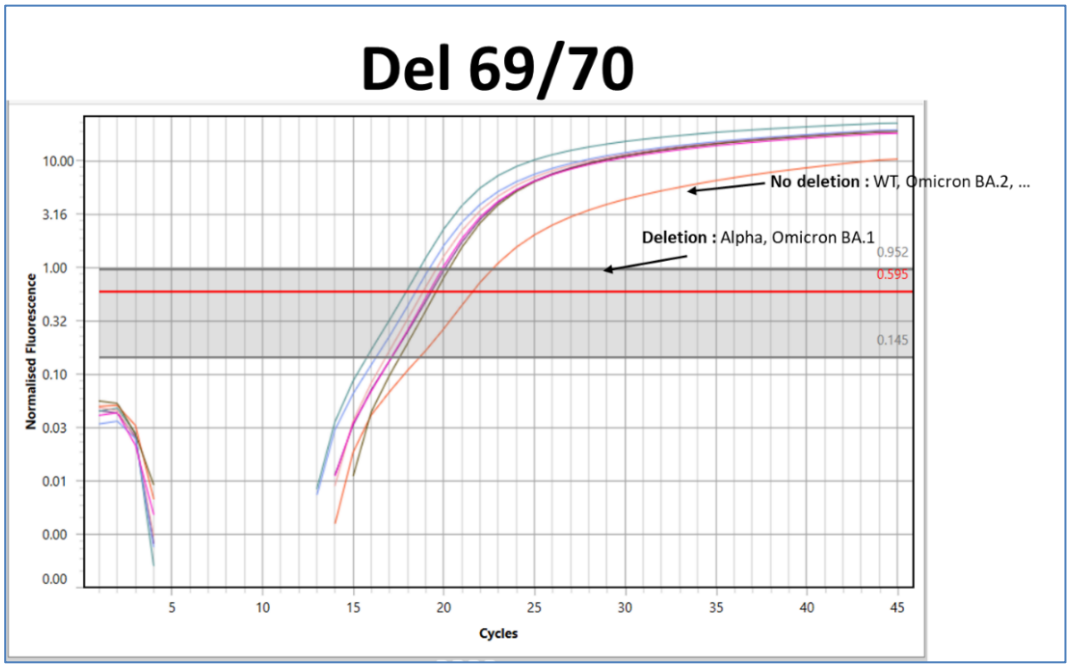



*Figure 4: Amplification curves for the deletion 69-70 with the qPCR Mic instrument (Well #1)*

## Mutations in E484 (Well #2)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **> 63** | FAM | The sample is positive for the E484K mutation | E484K |
| **>= 59 – 63** | FAM | The sample is most likely positive for the E484A mutation | E484A |
| **< 59** | FAM | The sample is negative for the E484K mutation | E484E (wild type) |
| **No peaks** | FAM | Not possible to determine genotype | Uninterpretable |

*Table 4: Analysis of melting peaks results for mutations in E484 with the qPCR Mic instrument (Well #2)*

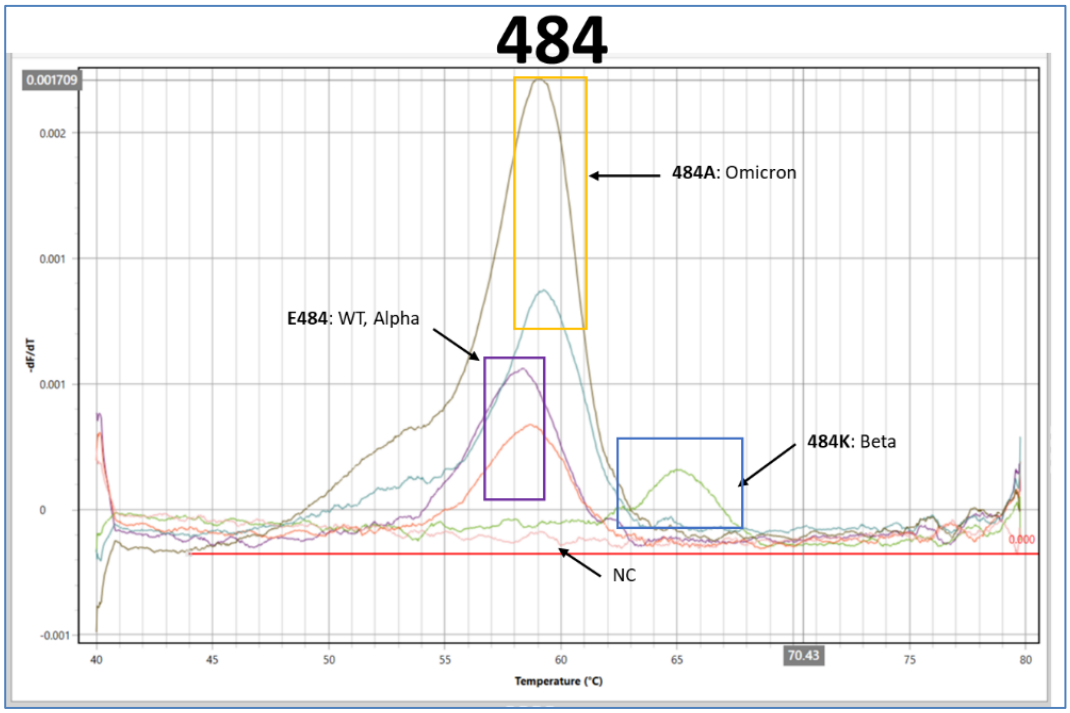



*Figure 5: Melting curves for the E484K target (Well #2) with the qPCR Mic instrument*

## Summary analysis table

| | N | 69–70del | 417N | 417T | 452R | 484K | 484A |
|---|---|---|---|---|---|---|---|
| **Well** | 1 / 2 | 1 | 1 | 1 | 1 | 2 | 2 |
| **Dye reporter** | HEX | ROX | Cy5 | Cy5 | FAM | FAM | FAM |
| **Value** | Ct | Ct | Tm | Tm | Tm | Tm | Tm |
| **Alpha (UK)** | **>10** | **N/A** | 66 – 68.5°C | 66 – 68.5°C | 60 – 64°C | <59 | <59 |
| **Beta (SA)** | **>10** | **>10** | **>=69°C** | 66 – 68.5°C | 60 – 64°C | **>=63°C** | <59 |
| **Gamma (BR)** | **>10** | **>10** | 66 – 68.5°C | **<66°C** | 60 – 64°C | <59 | <59 |
| **Delta (IN)** | **>10** | **>10** | **(>=69°C)*** | 66 – 68.5°C | **>=66°C** | <59 | <59 |
| **Omicron (BA.1)** | **>10** | **N/A** | **>=69°C** | 66 – 68.5°C | 60 – 64°C | <59 | **>=59 – 63°C** |
| **BA.2** | **>10** | **>10** | **>=69°C** | 66 – 68.5°C | 60 – 64°C | <59 | **>=59 – 63°C** |

*Table 5: Summary of expected value (Ct or Tm) per Assay target with the qPCR Mic instrument*

Important: (*) K417N is missing for strains B.1.617.2: Tm are measured between 66°C and 68.5°C.

## Quality controls

Controls that are provided with the ***UltraGene Assay SARS-CoV-2 VOC Screening & Determination V1.X*** (RUO) are listed below:

a) A "no template" (negative) control (NTC) consisting of Water (molecular grade) is used and is needed to detect cross-contamination during all reaction steps. The NTC is used during the PCR and is used to determine validity of the test run.
b) A positive template control [(ZeptoMetrix SARS-CoV-2 (Isolate: USA-WA1/2020) Culture Fluid (Heat Inactivated) (Catalog# 0810587CFHI)], diluted into nuclease-free water is needed to ensure the rRT-PCR reaction setup and reagent integrity and is used to validate the rRT-PCR plate results. The positive control is used during PCR.
c) An additional primer/probe set labeled with a distinct fluorophore (FAM) is included in the Primer/Probe Mix that targets the SARS-CoV-2 Nucleocapsid gene (S) that is present in positive specimens.

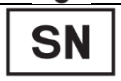
## Limitations

- **For Research Use Only (RUO). Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.**
- Laboratories maybe required to report all test results to the appropriate public health authorities.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may affect the test performance.
- A false negative result may occur if a specimen is improperly stored or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- If the virus mutates in the test target region, SARS-CoV-2 RNA may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- False-positive results may arise from cross contamination during specimen handling, preparation, assay set-up or product handling.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic, immunosuppressant drugs or cold medications have not been evaluated.

## Product quality control

In accordance with ABL's Quality Management System, each lot of the assay is tested against predetermined specifications to ensure consistent product quality. Certificates of Analysis are available upon request.

## Symbols

| | | | |
|---|---|---|---|
|  <N> | Contains reagents enough for <N> reactions |  | Consult instructions for use |
|  | Caution |  | Negative control |
|  | Catalog number |  | Positive control |
|  | Use by |  | Temperature limitation |
|  | Manufacturer |  | Serial Number |
|  | Country of manufacture with a date of manufacture | **R*n*** | R is for revision of the Instructions for Use (IFU) and n is the revision number |
|  | Distributor | | |

## Contact Information

For technical assistance and more information, please see our Technical Support Center at Online: https://ablsa.odoo.com/fr_FR/issue; Email: support-diag@ablsa.com; Call +339 7017 0300 Or contact your ABL Field-Application Specialist or your local distributor. For up-to-date licensing information or product-specific disclaimers, see the respective ABL Assay User Guide. ABL User Guides are available at **www.ablsa.com/ifu** or can be requested from ABL Technical Services or your local distributor.

## Manufacturer

Advanced Biological Laboratories (ABL) S.A.

52-54 avenue du X Septembre, 2550 Luxembourg, Luxembourg

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the device. The information in this guide is subject to change without notice. DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, ABL (S.A) AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.



Version 1.1

Effective date: 3rd February 2022

# Annex A – Use with CFX96 C1000 Instrument

## PCR cycling conditions

Thermal cycling program Set up the cycling program for the laboratory validated real-time PCR instrument.

We give here example with the following real-time PCR instrument:

- C1000 Dx Thermal Cycler (Bio-Rad, Catalog # 1841000-IVD with CFX96-Dx ORM software version 3.1) (C1000 CFX96)

Note: Ensure that instrument has been checked and calibrated according to the manufacturer's recommendations. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.

| Stage | Temperature (°C) | Time (min:sec) |
|---|---|---|
| **1** | 55 | 10:00 |
| **2** | 95 | 03:00 |
| **3** | 94 | 00:15 |
| **4** | 60 | 00:45 |
| | +plate Read | |
| **5** | GOTO 3. | 44 more times |
| **6** | 95 | 01:00 |
| **7** | 40 | 01:00 |
| **8** | Melt curve 40.0 to 80.0 °C increment | 0.5 °C |
| | For 0:05 + plate Read | |
| | END | |

*Table 7: PCR Cycling program for the C1000 CFX96 instrument (For melting curve: 2 reading/C°)*

## Selection of fluorescence channel

Please refer to the C1000 CFX96 instructions for use for the setup of the fluorescence channels FAM, HEX, ROX and Cy5 for the SARS-CoV-2 S and N targets.

## Results analysis per assay targets

### Mutations in K417 (Well #1)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **>= 64** | Cy5 | The sample is most likely positive for the K417N mutation | K417N |
| **61.5 – 63.5** | Cy5 | The sample is negative for the K417N mutation | K417K (wild type) |
| **< 60** | Cy5 | The sample is negative for the K417N mutation, but most likely contain a different mutation in the probe area | Other than K417N mutation (K417T) |
| **No peaks** | Cy5 | Not possible to determine genotype | Uninterpretable |

*Table 8: Analysis of melting peaks results for mutations in K417 with the C1000 CFX96 instrument (Well #1)*

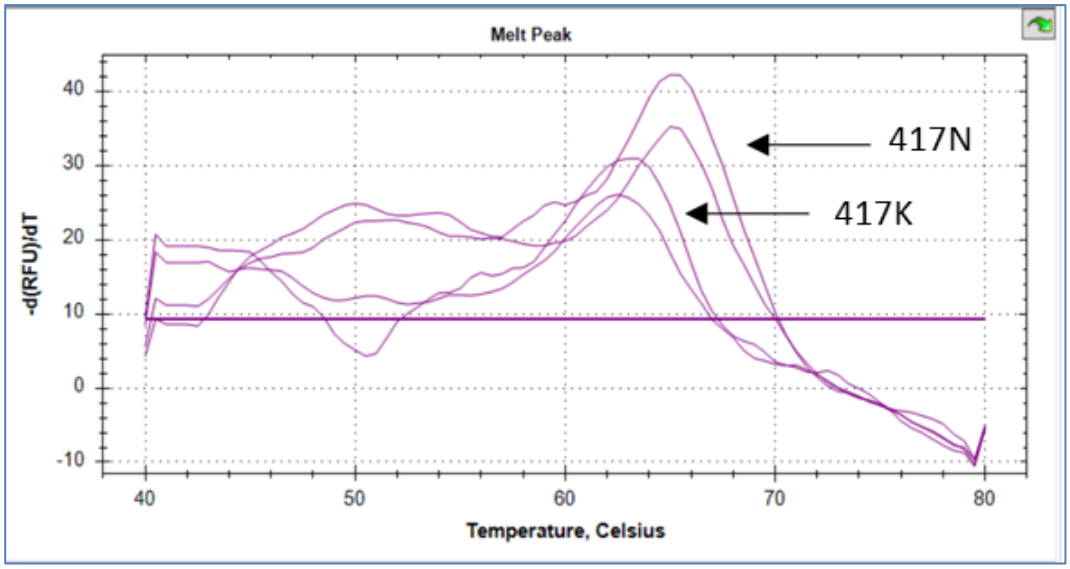



*Figure 6: Melting curves for the K417N target with C1000 CFX96 instrument (Well #1)*

## Mutations in L452 (Well #1)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **>= 64** | FAM | The sample is positive for the L452R mutation | L452R |
| **60 – 63** | FAM | The sample is negative for the L452R mutation | L452 Wild Type |
| **< 60** | FAM | The sample is negative for the L452R mutation, but most likely contain a different mutation in the probe area. | Other than L452R mutation |
| **No peaks** | FAM | Not possible to determine genotype | Uninterpretable |

*Table 9: Analysis of melting peaks results for mutations in L452 with C1000 CFX96 instrument (Well #1)*

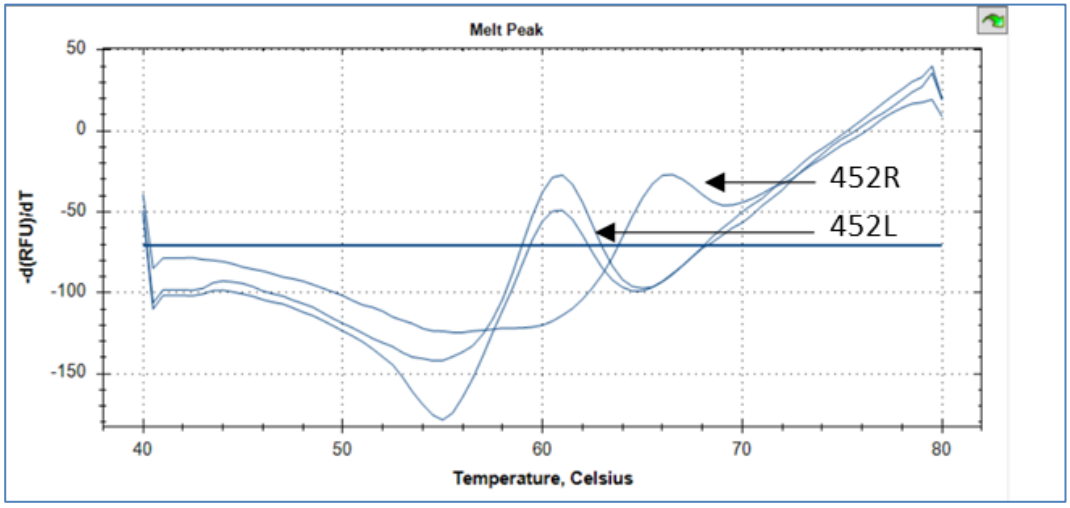



*Figure 7: Melting curves for the L452R target with C1000 CFX96 instrument (Well #1)*

## Deletion 69-70 (Well #1)

| Ct value | Channel | Analysis | Reporting |
|---|---|---|---|
| **N/A** | ROX | The sample is positive for the deletion | Presence of the deletion 69-70 |
| **> 10** | ROX | The sample is negative for the deletion | Absence of the deletion 69-70 |

*Table 10: Analysis of Ct value results for deletion 69-70 with the C1000 CFX96 instrument (Well #1)*

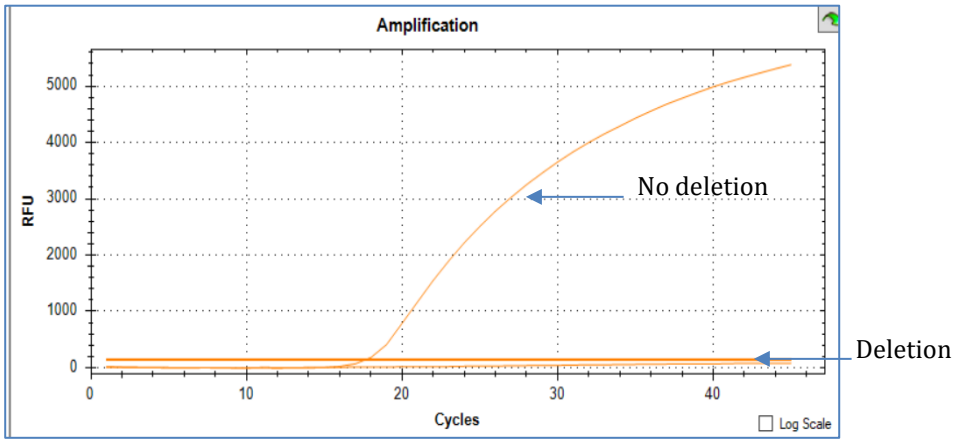



*Figure 8: Amplification curves for the deletion 69-70 with the C1000 CFX96 instrument (Well #1)*

## Mutations in E484 (Well #2)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **> 63** | FAM | The sample is positive for the E484K mutation | E484K |
| **57.5 – 58.5** | FAM | The sample is most likely positive for the E484A mutation | E484A |
| **54 – 57** | FAM | The sample is negative for the E484K mutation | E484E (wild type) |
| **< 54** | FAM | The sample is negative for the E484K mutation, but most likely contain a different mutation in the probe area | Other than E484K mutation |
| **No peaks** | FAM | Not possible to determine genotype | Uninterpretable |

*Table 11: Analysis of melting peaks results for mutations in E484 with the C1000 CFX96 instrument (Well #2)*

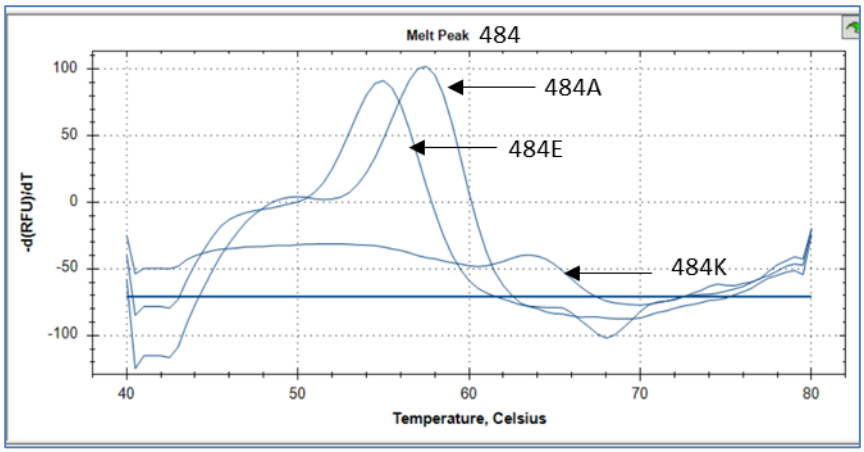



*Figure 9: Melting curves for the E484K target (Well #2) with the C1000 CFX96 instrument*

### Summary analysis table

| | N | 69–70del | 417N | 417T | 452R | 484K | 484A |
|---|---|---|---|---|---|---|---|
| **Well** | 1 / 2 | 1 | 1 | 1 | 1 | 2 | 2 |
| **Dye reporter** | HEX | ROX | Cy5 | Cy5 | FAM | FAM | FAM |
| **Value** | Ct | Ct | Tm | Tm | Tm | Tm | Tm |
| **Alpha (UK)** | **>10** | **N/A** | 61.5 – 63.5°C | 61.5 – 63.5°C | 60 – 63°C | 54 – 57°C | 54 – 57°C |
| **Beta (SA)** | **>10** | **>10** | **>=64°C** | 61.5 – 63.5°C | 60 – 63°C | **>63°C** | 54 – 57°C |
| **Gamma (BR)** | **>10** | **>10** | 61.5 – 63.5°C | **<60°C** | 60 – 63°C | **>63°C** | 54 – 57°C |
| **Delta (IN)** | **>10** | **>10** | **(>=64°)*C** | 61.5 – 63.5°C | **>=64°C** | 54 – 57°C | 54 – 57°C |
| **Omicron (BA.1)** | **>10** | **N/A** | **>=64°C** | 61.5 – 63.5°C | 60 – 63°C | 54 – 57°C | **57.5-58.5°C** |
| **BA.2** | **>10** | **>10** | **>=64°C** | 61.5 – 63.5°C | 60 – 63°C | 54 – 57°C | **57.5-58.5°C** |

*Table 8: Summary of expected value (Ct or Tm) per Assay target with the C1000 CFX96 instrument*

Important: (*) K417N is missing for strains B.1.617.2: Tm are measured between 61.5°C and 63.5°C.

# Annex B – Use with QuantStudio 5 Instrument

## PCR cycling conditions

Thermal cycling program Set up the cycling program for the laboratory validated real-time PCR instrument.

We give here example with the following real-time PCR instrument:

- QuantStudio 5 Dx Real-Time PCR Instrument (96-Well 0.2mL Block) (Applied Biosystems, Catalog #A47326, Design & Analysis Software 1.02 / Firmware Version 1.0.3)

Note: Ensure that instrument has been checked and calibrated according to the manufacturer's recommendations. Please refer to relevant manufacturer's Instructions for Use (IFU) to proceed with the instrument.


*Figure 10: PCR Cycling program for the QS5 DX qPCR instrument*

## Selection of fluorescence channel

Please refer to the qPCR QS5 DX instructions for use for the setup of the fluorescence channels FAM, HEX, ROX and Cy5 for the SARS-CoV-2 S and N targets.

## Results analysis per assay targets

## Mutations in K417 (Well #1)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **>= 64** | Cy5 | The sample is most likely positive for the K417N mutation | K417N |
| **61 – 63.5** | Cy5 | The sample is negative for the K417N mutation | K417K (wild type) |
| **< 61** | Cy5 | The sample is negative for the K417N mutation, but most likely contain a different mutation in the probe area | Other than K417N mutation (K417T) |
| **No peaks** | Cy5 | Not possible to determine genotype | Uninterpretable |

*Table 12: Analysis of melting peaks results for mutations in K417 with the QS5 DX (Well #1)*

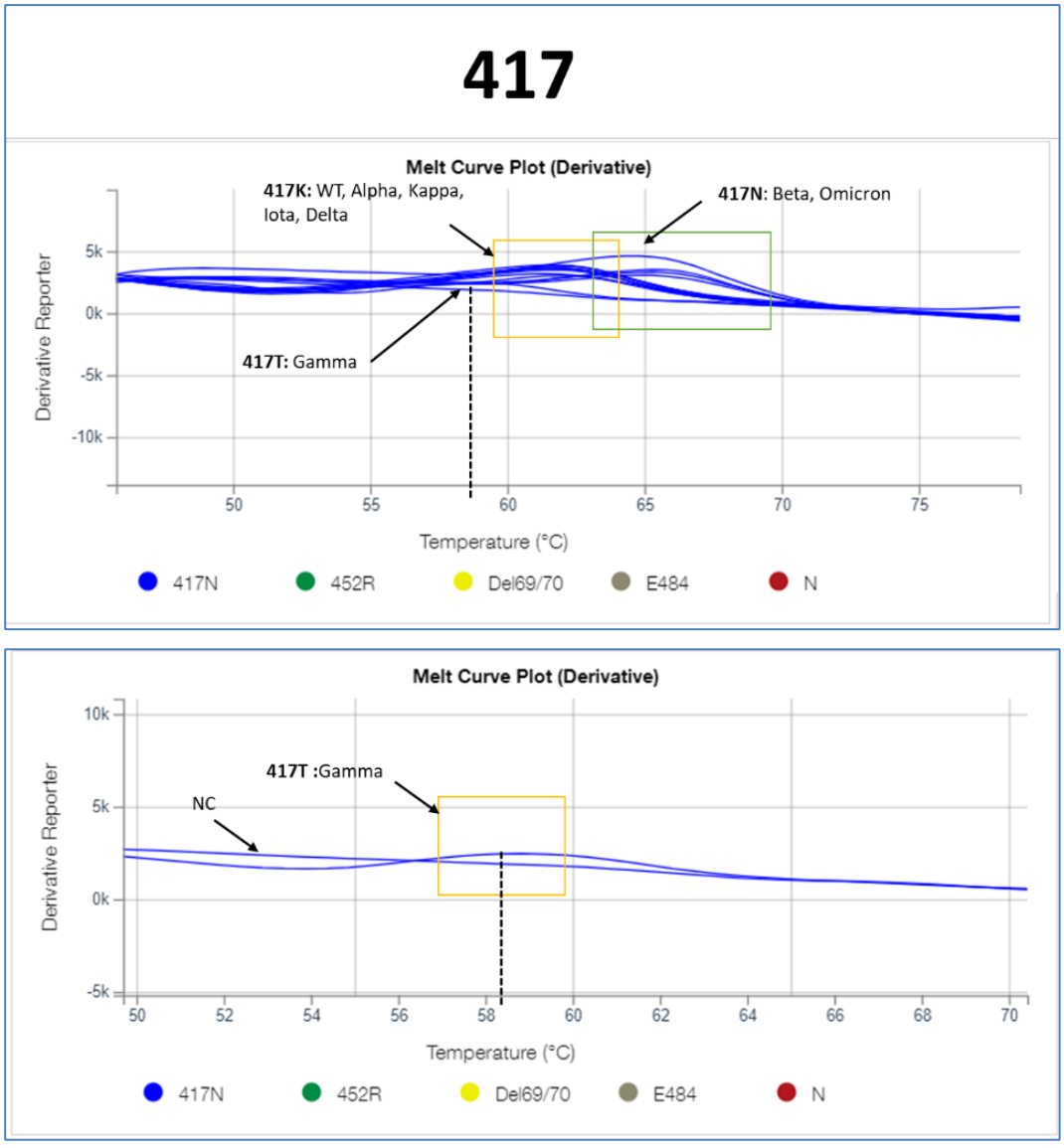



*Figure 11: Melting curves for the K417N target with the QS5 DX (Well #1)*

## Mutations in L452 (Well #1)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **>= 65** | FAM | The sample is positive for the L452R mutation | L452R |
| **60 – 62** | FAM | The sample is negative for the L452R mutation | L452 Wild Type |
| **< 60** | FAM | The sample is negative for the L452R mutation, but most likely contain a different mutation in the probe area. | Other than L452R mutation |
| **No peaks** | FAM | Not possible to determine genotype | Uninterpretable |

*Table 13: Analysis of melting peaks results for mutations in L452 with QS5 DX (Well #1)*

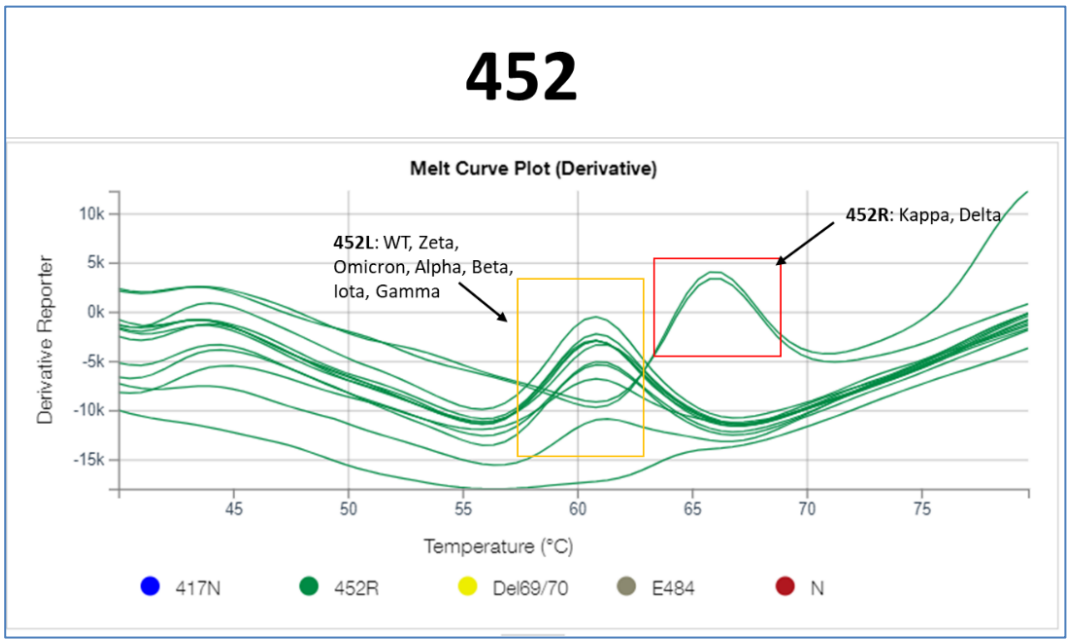



*Figure 12: Melting curves for the L452R target with QS5 DX (Well #1)*

## Deletion 69-70 (Well #1)

| Ct value | Channel | Analysis | Reporting |
|---|---|---|---|
| **N/A** | ROX | The sample is positive for the deletion | Presence of the deletion 69-70 |
| **> 10** | ROX | The sample is negative for the deletion | Absence of the deletion 69-70 |

*Table 14: Analysis of Ct value results for deletion 69-70 with the QS5 DX (Well #1)*

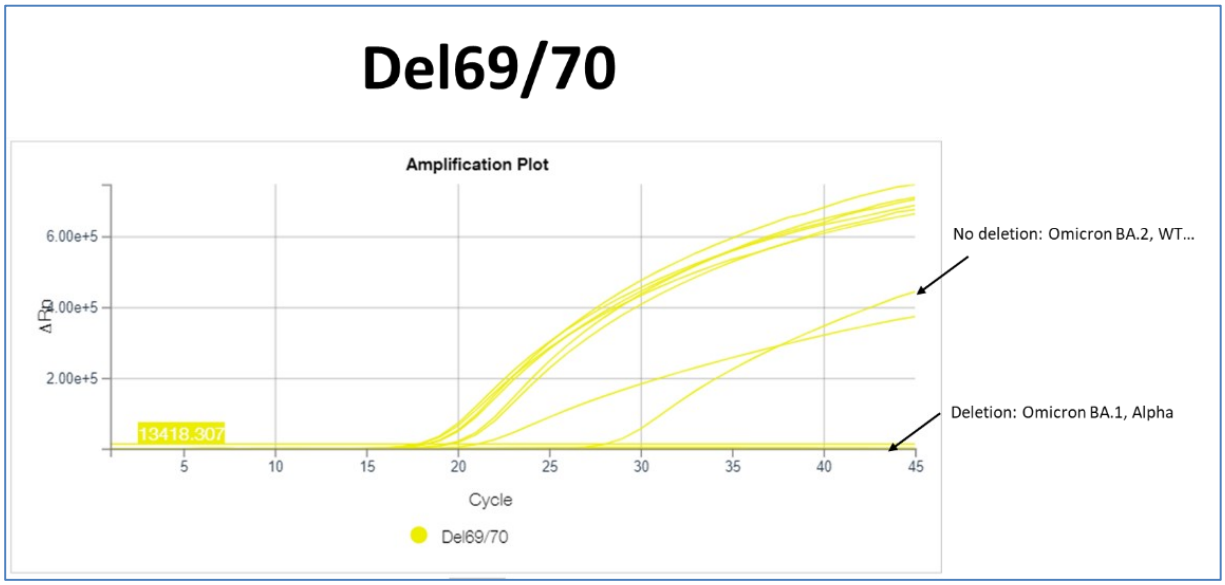



*Figure 13: Amplification curves for the deletion 69-70 with the QS5 DX (Well #1)*

## Mutations in E484 (Well #2)

| Melting peak (°C) | Channel | Analysis | Reporting |
|---|---|---|---|
| **> 63** | FAM | The sample is positive for the E484K mutation | E484K |
| **>= 57 – 58** | FAM | The sample is most likely positive for the E484A mutation | E484A |
| **>= 58.5 – 59** | FAM | The sample is most likely positive for the E484Qmutation | E484Q |
| **<= 56.5** | FAM | The sample is negative for the E484K mutation | E484E (wild type) |
| **No peaks** | FAM | Not possible to determine genotype | Uninterpretable |

*Table 15: Analysis of melting peaks results for mutations in E484 with the QS5 DX (Well #2)*

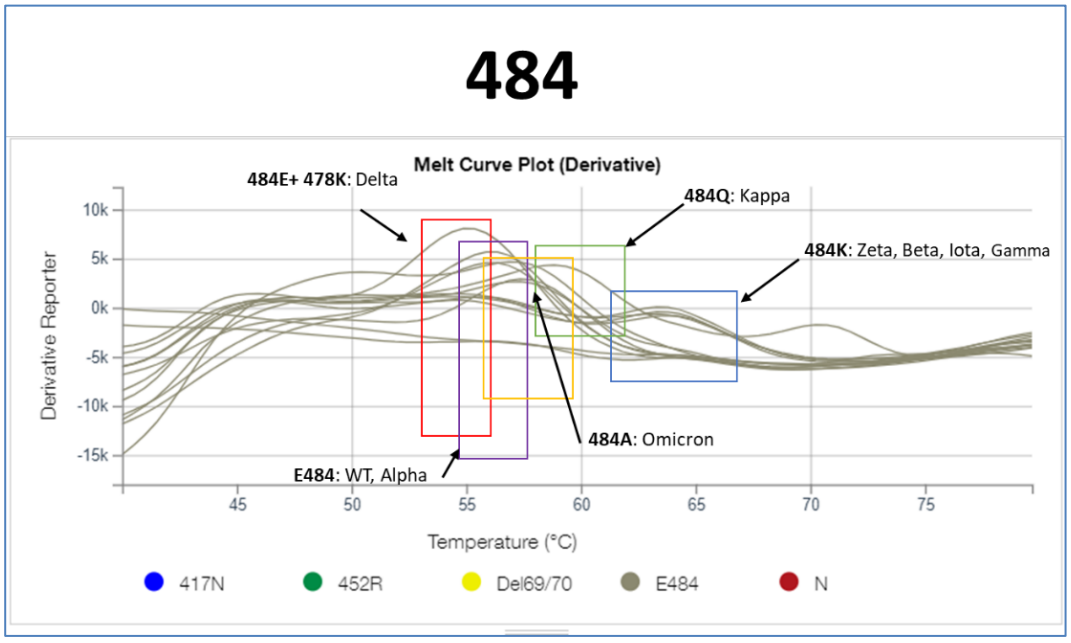



*Figure 14: Melting curves for the E484K target (Well #2) with the QS5 DX*

### Summary analysis table

| | N | 69–70del | 417N | 417T | 452R | 484K | 484A |
|---|---|---|---|---|---|---|---|
| **Well** | 1 / 2 | 1 | 1 | 1 | 1 | 2 | 2 |
| **Dye reporter** | HEX | ROX | Cy5 | Cy5 | FAM | FAM | FAM |
| **Value** | Ct | Ct | Tm | Tm | Tm | Tm | Tm |
| **Alpha (UK)** | **>10** | **N/A** | 61 – 63.5°C | 61 – 63.5°C | 60 – 62°C | <=56.5°C | <=56.5°C |
| **Beta (SA)** | **>10** | **>10** | **>=64°C** | 61 – 63.5°C | 60 – 62°C | **>=63°C** | <=56.5°C |
| **Gamma (BR)** | **>10** | **>10** | 61 – 63.5°C | **<61°C** | 60 – 62°C | <=56.5°C | <=56.5°C |
| **Delta (IN)** | **>10** | **>10** | **(>=64°C)*** | 61 – 63.5°C | **>=65°C** | <=56.5°C | <=56.5°C |
| **Omicron (BA.1)** | **>10** | **N/A** | **>=64°C** | 61 – 63.5°C | 60 – 62°C | <=56.5°C | **>=57 – 58°C** |
| **BA.2** | **>10** | **>10** | **>=64°C** | 61 – 63.5°C | 60 – 62°C | <=56.5°C | **>=57 – 58°C** |

*Table 16: Summary of expected value (Ct or Tm) per Assay target with the QS5 DX*

Important: (*) K417N is missing for strains B.1.617.2: Tm are measured between 61°C and 63.5°C.