Primerdesign Ltd

SNPsig® SARS-CoV-2 (20I/501Y.V1)

UK variant

SNPsig® real-time PCR
SARS-CoV-2 mutation detection/
allelic discrimination kit

96 tests

For general laboratory and research use only

Kits by Primerdesign

Kit contents

- **SARS-CoV-2 20I/501Y.V1 genotyping primer/probe mix (96 reactions BROWN)**
  FAM and HEX labelled
- **Wild-type positive control template (RED)**
- **Mutant positive control template (RED)**
- **RNase/DNAse free water (WHITE)**
  for resuspension of primer/probe mix
- **Template preparation buffer (YELLOW)**
  for resuspension of positive control templates
- **oasig® Lyophilised OneStep 2X RT-qPCR Mastermix (RED)**
  contains complete Onestep RT-qPCR mastermix
- **oasig® resuspension buffer (BLUE)**
  for resuspension of the lyophilized mastermix

Reagents and equipment to be supplied by the user

Real-time PCR Instrument
Must be able to read fluorescence through FAM and HEX channels (plus Cy5 if using optional internal control)

Pipettes and tips

Vortex

Centrifuge

Suitable qPCR 96W plates or qPCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20ºC on arrival. Primerdesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilised components have been resuspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Suitable sample material

SNPsig® SARS-CoV-2 UK SNP is intended for use as a **reflex test** only. Thus, a primary confirmation test for SARS-CoV-2 would be carried out using suitable methodology, and the extracted RNA from patient samples (or any material suited for PCR amplification) thereafter applied to this test. Please ensure that the samples are suitable in terms of purity, concentration, and RNA integrity. Always run at least one negative control with the samples. To prepare a negative control, replace the template RNA sample with RNase/DNAse free water.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign SNPsig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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Introduction

The Novel Coronavirus Disease 2019 (COVID-19) pandemic, caused by SARS-CoV-2 virus, represents a major threat to health. COVID-19 has resulted in widespread morbidity and mortality. SARS-CoV-2 is known to have infected more than 90 million people (1). As the virus has spread across the world, mutations have arisen resulting in divergent clusters (clades) with different prevalence in different geographic regions (2,3).

One major clade to be identified is 20I/501Y.V1, also known as VOC202012/01 and B.1.1.7. This variant has been identified as approximately 70% more transmissible (4). This variant contains the N501Y replacement in the spike protein and has been shown to increase ACE2 binding (5). This variant has been seen with a 69-70 nucleotide deletion which has been shown to impact performance of some diagnostic PCR assays using an S gene target (6). In addition, this variant contains P681H which is located near a furin cleavage site and is believed to contribute to the increase in transmissibility (7,8). Recent independent analyses suggest that there might be a realistic possibility that infection with VOC 20I/501Y.V1 is associated with an increased risk of death compared to infection with non-VOC viruses (9).

20I/501Y.V1 has now been detected in 60 countries, comprising all six WHO regions to date (10). Currently, authorities are using the occurrence of S gene target failure (SGTF) as a proxy for this strain. However, given the shared nature of these mutations and rising evidence for convergent evolution, tests are needed to define specific strains. Novacyt performed a thorough bioinformatic investigation to find unique identifiers to the 20I/501Y.V1 variant and selected the optimal Single-Nucleotide Polymorphism (SNP) to identify this variant (UK SNP). Targeting of UK SNP is 100% specific for this strain and will allow targeted testing of positive SARS-CoV-2 samples to find the 20I/501Y.V1 variant.

Principles of the test

Genotyping by real-time PCR using hydrolysis probes

Each genotyping primer/probe mix contains two labelled probes homologous to the two genotypes under investigation. During qPCR amplification of the reverse-transcribed target RNA, the probes will compete for binding across the variant region. The probe that is 100% homologous to the binding site will preferentially bind and give a fluorescent signal as PCR proceeds. It follows that the wild-type sequence will give a strong amplification plot through one channel whilst giving a very weak signal through the alternative channel. Variant samples will give an exactly inverse result. Most hardware platforms can perform this analysis automatically. The SNPsig® assays are compatible with all qPCR instruments capable of detecting fluorescence in FAM and HEX (plus Cy5 if using internal control) emission channels, including selected genesig® family instruments.

Positive controls

The kit contains positive control templates for each of the two genotypes. These can be run as parallel samples to give control signals for each genotype. In order to provide good positive control data that is directly relevant to the samples under test, the positive control template should be used at a similar copy number to the sample RNA so instructions are given to dilute the positive controls accordingly. Positive control templates are a potential contamination risk to subsequent tests so must be handled carefully.

Negative control
To confirm the absence of contamination, a negative control reaction should be included every time the kit is used. In this instance, the RNase/DNAse free water should be used instead of template RNA. A negative result in FAM and HEX channels indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained, this indicates contamination, therefore the sample results will be invalid and the run should be repeated. Possible sources of contamination should first be explored and removed.

Master mix compatibility
oasig® Lyophilised OneStep 2X RT-qPCR Master Mix contains the enzyme, nucleotides, buffers and salts at precisely the correct concentration for this application. The annealing temperatures of the primer and probe have been carefully calibrated and any change in the reaction buffer can significantly alter the performance of the assay. For this reason, Primerdesign can only guarantee accurate genotyping results when oasig® Lyophilised OneStep 2X RT-qPCR Master Mix is used.

Resuspension Protocol

To minimize the risk of contamination with foreign RNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps. The positive control template is a significant contamination risk and should therefore be pipetted after negative control and sample wells.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNAse free water supplied, according to the table below.**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - Resuspend in water	Volume
SARS-CoV-2 20I/501Y.V1 primer/probe mix (BROWN)	110 µl

3. **Resuspend the mastermix in oasig resuspension buffer supplied, according to the table below.**

Component - Resuspend in resuspension buffer	Volume
oasig® Lyophilised OneStep 2X RT-qPCR Mastermix (RED)	525 µl

4. **Resuspend the positive control templates in the template preparation buffer supplied, according to the table below.**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - Resuspend in template preparation buffer	Volume
Wild-type Positive Control Template (RED) *	500 µl
Mutant Positive Control Template (RED) *	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

qPCR detection protocol

If using optional internal control, refer to page 11 for protocol amendments.

1. **Prepare a complete genotyping reaction mix for the primer/probe mix according to the table below:**
   Include sufficient reactions for all samples, positive and negative controls.

Component	Volume
oasig® OneStep 2X RT-qPCR Master Mix Lyophilised	10 µl
SARS-CoV-2 20I/501Y.V1 primer/probe mix (BROWN)	1 µl
RNase/DNAse free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Prepare RNA templates for each of your samples.**
   As this is a reflex test, the RNA obtained from the confirmatory primary assay for SARS-CoV-2 should be used.

4. **Pipette 5µl of RNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNAse free water. The final volume in each well is 20µl.

5. **Dilute each positive control template in template preparation buffer.**
   The positive control templates must each be diluted according to the table below:

Copies/reaction	Positive control dilution factor
10^4	1:100

6. **Pipette 5µl of each positive control DNA, according to your experimental plate set up.**

qPCR amplification protocol

The following protocol is recommended for optimum resolution between genotypes:

Protocol for oasig® OneStep 2X RT-qPCR Master Mix

	Step	Time	Temp
	Reverse transcription	10 min	55 °C
	Enzyme activation	2 min	95 °C
Cycling x45	Denaturation	10s	95 °C
	Annealing and extension (DATA COLLECTION) *	60s	60 °C

* Fluorogenic data should be collected during this step through the **FAM** and **HEX** channels

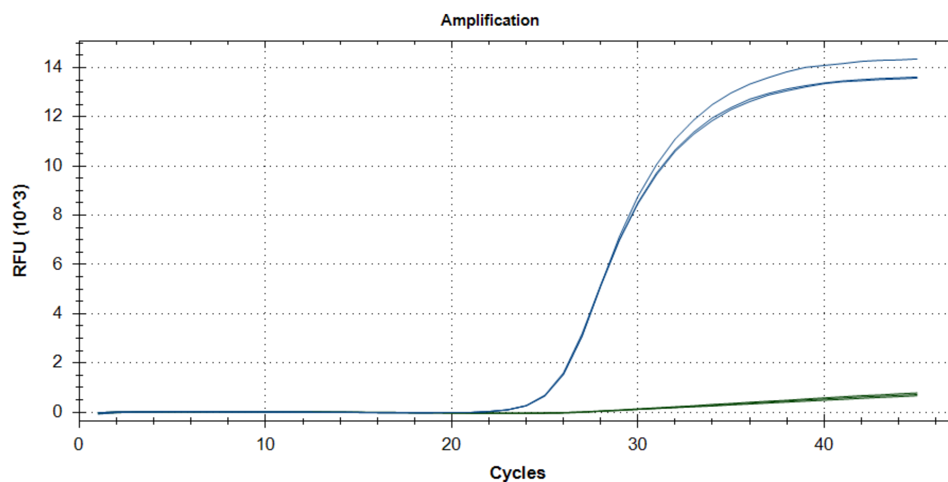
Interpretation of results

The wild-type probe is labelled to read through the FAM channel whilst the mutant probe is labelled to read through the HEX channel. On wild-type sequences the FAM channel will give a strong amplification plot and the HEX channel none or very low detection. The signals are reversed on mutant samples. Cq values >40 should be disregarded and counted as inconclusive.

If using an internal control (see protocol below), then the Cy5 channel should be consulted where the test is negative in both the FAM and HEX channels. If neither HEX or FAM amplify, but Cy5 channel is observed, then the reaction worked, and the test is a true negative. If no Cy5 amplification is observed, then the test failed.

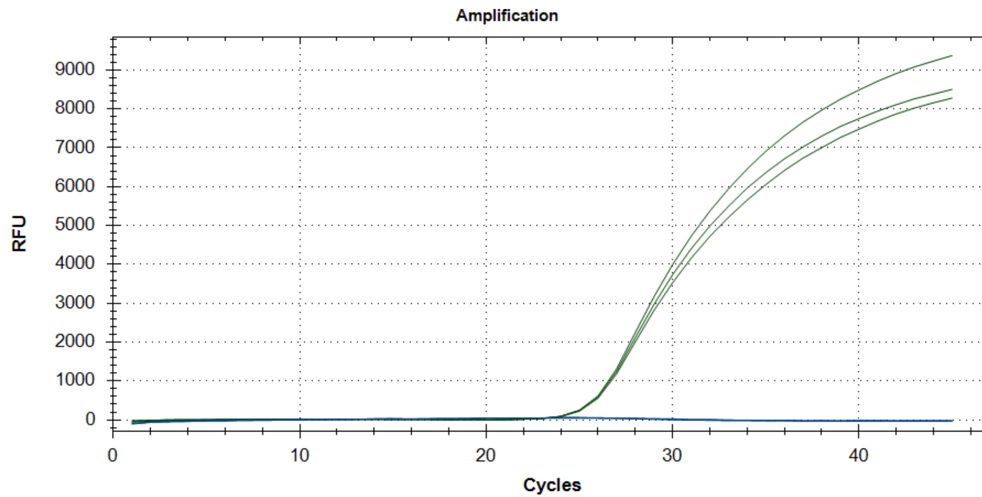
Sample data

Wild Type sample (**WT signal**, **Mutant signal**)



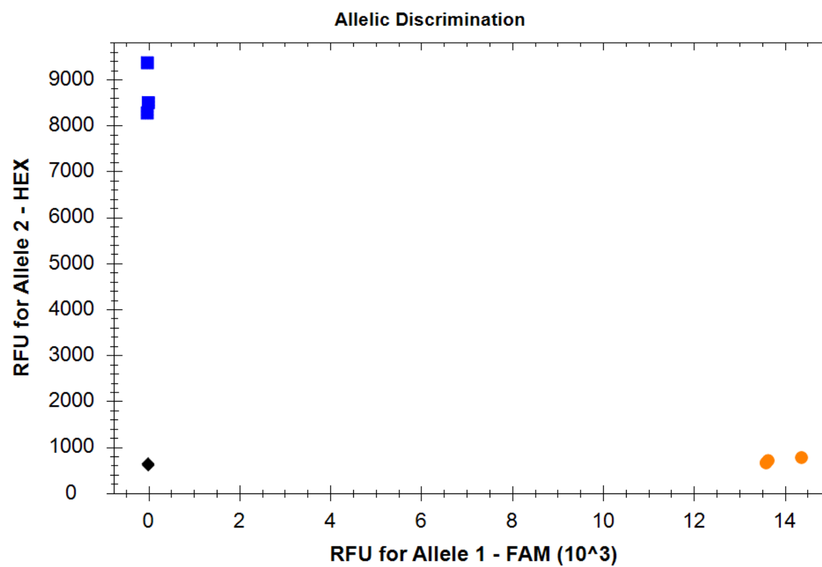
Amplification in the FAM (Blue) channel with no amplification in the HEX (green) channel indicates the presence of wild type template only.

Variant RNA sample (WT signal, Mutant signal)



Amplification in the HEX (green) channel with no amplification in the FAM (blue) channel indicates the presence of mutant template only.

The raw data above can best be visualised by using a cluster analysis; plotting the end point fluorescence data from the FAM channel on one axis and the end point fluorescence data from the HEX channel on the other axis. Most qPCR software platforms will perform this analysis automatically so follow the manufacturer's instructions for your software. The data are quickly resolved into clusters corresponding to the wild-type, and mutant variant samples.



Optional: Use of Internal Control (CY5)

Internal RNA control (CY5) is available as a separate product, compatible with this kit (Product Code: Z-INT-RNA-CY5-LL).

To confirm the occurrence of PCR, an internal control reaction can be used. Successful real-time PCR amplification of the internal control indicates that PCR inhibitors are not present at a high concentration. An internal control primer/probe mix labelled with the CY5 fluorophore can be used with this kit to detect the exogenous RNA template following cDNA synthesis using qPCR. The primers and CY5 probe allow detection of the control cDNA alongside the target cDNA in a multiplex reaction. Amplification of the control cDNA does not interfere with detection of the target gene even when the target gene is present at low copy number. If a positive result is obtained, this indicates that the reaction was successful and that any negative result for the primary reaction is a true negative.

Resuspension Protocol

To minimize the risk of contamination with foreign RNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer and probe mix is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNAse free water supplied, according to the table below.**
   To ensure complete resuspension, vortex the tube thoroughly.

Component - Resuspend in water	Volume
Internal Control primer/probe mix CY5 (BROWN)	165 µl

3. **Resuspend internal control in 500 µl Template Preparation Buffer.**

Component - Resuspend in template preparation buffer	Volume
Internal Control RNA template (BLUE)*	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

4. **Dilute the resuspended internal control template 1:40 (e.g. adding 10 µl internal control template to 390 µl Template Preparation Buffer).**

5. **Prepare a complete genotyping reaction mix including the internal control primer/probe mix and the diluted internal control template according to the table below:**
   Include sufficient reactions for all samples, positive and negative controls.

Component	Volume
oasig® OneStep 2X RT-qPCR Master Mix Lyophilised	10 µl
SARS-CoV-2 20I/501Y.V1 primer/probe mix (BROWN)	1 µl
Internal Control primer/probe mix (BROWN)	1 µl
1:40 dilution of Internal Control RNA template	1 µl
RNase/DNAse free water (WHITE)	2 µl
Final Volume	**15 µl**

6. **Follow qPCR amplification directions on page 8 with the exception that fluorogenic data should be collected through the FAM, HEX and Cy5 channels.**

References

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