Primerdesign Ltd

SNPsig® SARS-CoV-2 (20H/501Y.V2)

South Africa variant

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

96 tests

For general laboratory and research use only

Kit contents

- **SARS-CoV-2 (20H/501Y.V2) 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® OneStep 2X RT-qPCR Mastermix Lyophilised (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

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 SA 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).

In South Africa, one lineage has spread rapidly, quickly becoming the dominant strain, known as 20H/501Y.V2 or B.1.351 (4). This variant is characterized by eight lineage-defining mutations in the spike protein, including mutations at key residues (E484K, N501Y) which have been implicated in increased transmissibility and antibody evasion (5–7). In addition, the variant E484K raised concerns over the potential to impact vaccine efficacy (8–10). Rates of reinfection and potential for increased disease severity suggested for this variant have raised concern with public health bodies internationally.

As of 24th January 2021, 20H/501Y.V2 had been reported in 31 countries (11). Prevention of community outbreaks will need to be prioritised to ensure this lineage does not become more prevalent and a greater burden of COVID-19 in the health sector. Novacyt performed a thorough bioinformatic investigation to find unique identifiers to 20H/501Y.V2 variant and selected the optimal Single-Nucleotide Polymorphism (SNP) to identify this variant (SA SNP). Targeting of SA SNP is 100% specific for this strain and will allow targeted testing of positive SARS-CoV-2 samples to find the 20H/501Y.V2 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.

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. 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 indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples 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 (20H/501Y.V2) primer/probe mix (BROWN)	110 µl

3. **Resuspend the mastermix in 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

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 (20H/501Y.V2) 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 RNA 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

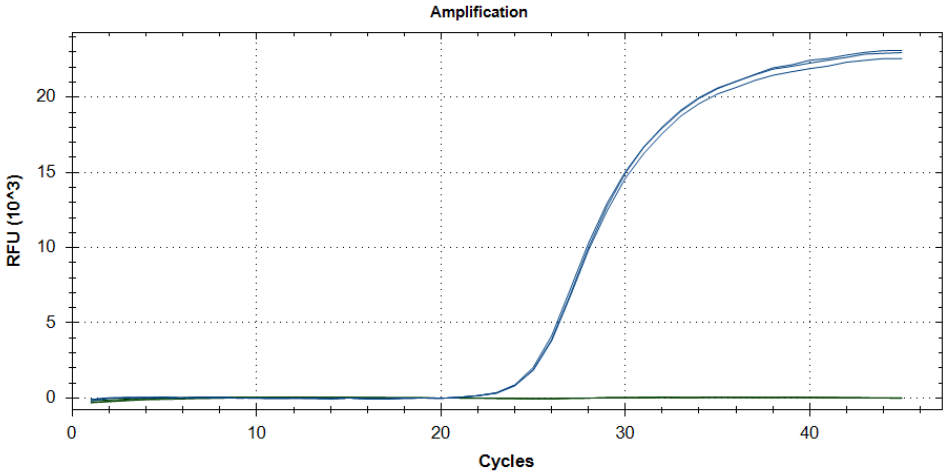
* Fluorogenic data should be collected during this step through the **FAM** and **HEX** channels

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.

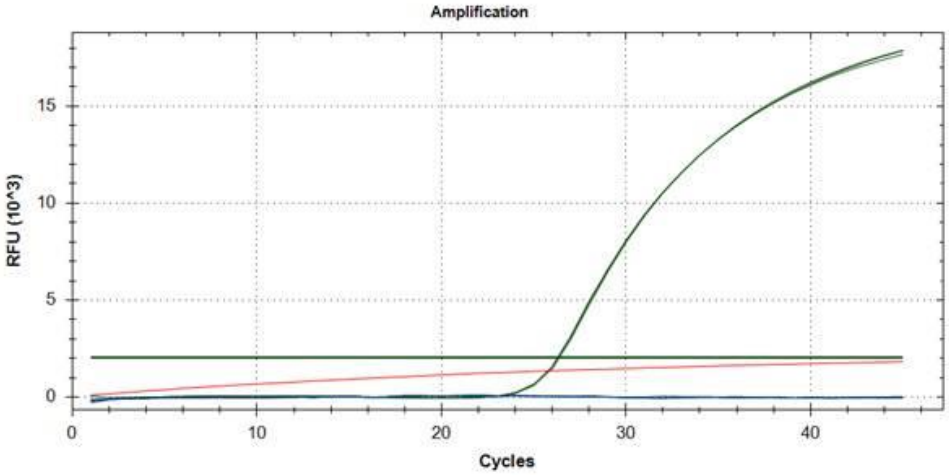
Sample data

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.

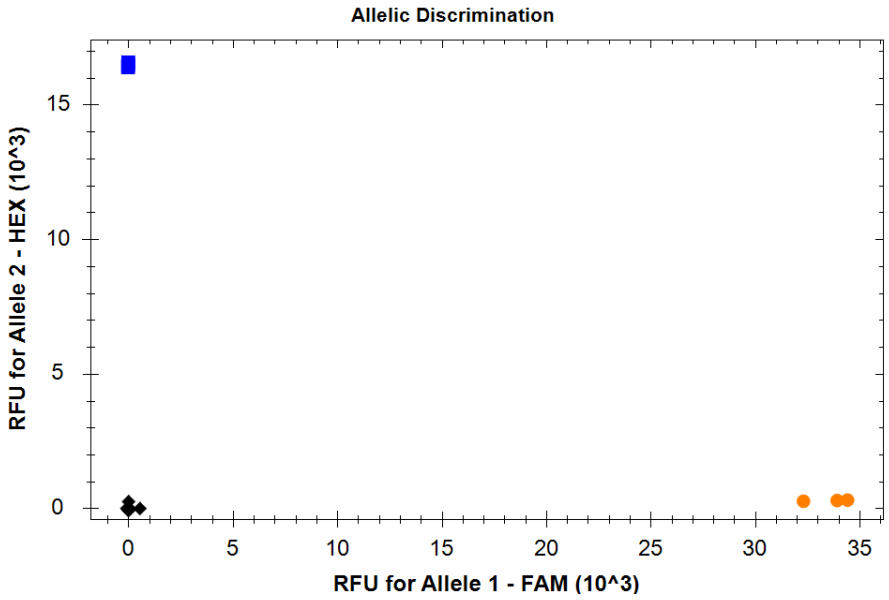
Variant RNA sample (**WT signal**, **Mutant signal**)
NTC HEX amplification shown in red



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

If the HEX background fluorescence is high, viewed as a low-level increase in fluorescence which is not sigmoid in shape (curve indicated in red), view the amplification plots in log scale and raise the threshold manually in order to exclude it.

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.



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