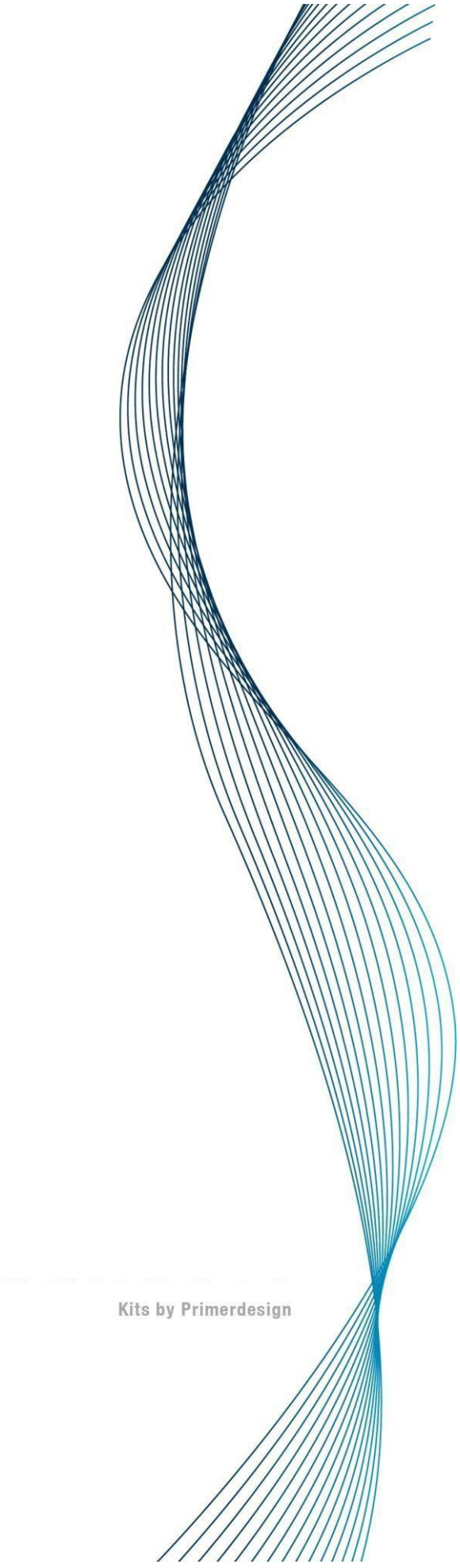
Primerdesign™ Ltd

genesig® SARS-CoV-2 Winterplex

genesig® real-time PCR assay

96 tests



For general laboratory and research use only

Detection of SARS-CoV-2, Influenza A, Influenza B, RSV A and RSV B
genesig® kit handbook. Path-genesig-SARS-CoV-2-Winterplex
Issue 1.00
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Kit contents

- **ORF1ab/S/M Primer & Probe Mix (including IEC primer/probe mix) (96 reactions BROWN)** FAM, HEX, ROX and Cy5 labelled
- **FluA/FluB/RSV Primer & Probe Mix (including IEC primer/probe mix) (96 reactions BROWN)** FAM, HEX, ROX and Cy5 labelled
- **genesig® Winterplex positive control template (RED)**
- **genesig® Winterplex internal extraction control (BLUE)**
- **RNase/DNAse free water (WHITE)**
  for resuspension of primer/probe mix
- **Template preparation buffer (YELLOW)**
  for resuspension of positive and internal extraction control templates
- **oasig® Lyophilised OneStep 2X RT-qPCR Mastermix (GOLD)**
  contains complete Onestep RT-qPCR mastermix
- **oasig® Mastermix 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, HEX/VIC, ROX and Cy5 channels

Pipettes and tips

Vortex

Centrifuge

Suitable qPCR 96 well 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

All kinds of sample material suited for PCR amplification can be used. 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

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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 100 million people. While SARS-CoV-2 is being tackled, other respiratory viruses are still circulating, and the Winterplex assay enables detection of Influenza A and Influenza B, and RSV A and B., in addition to a SARS-CoV-2 test that targets three distinct regions of the SARS-CoV-2 genome.

Principles of the test

Detection by real-time PCR using hydrolysis probes

The genesig® SARS-COV-2 Winterplex Real Time PCR assay is based on the standard hydrolysis probe system known as TaqMan® Technology. Real-Time PCR technology utilizes polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified RNA. The probes are labelled with fluorescent reporter and quencher dyes.

RNA is reverse transcribed to cDNA and subsequently amplified using a thermal cycler capable of detection in FAM, HEX/VIC, ROX and Cy5 channels. During PCR cycling, the probe anneals to a specific target sequence located between the forward and reverse primers. The probe is cleaved by the 5' nuclease activity of the Taq polymerase during the extension phase of the PCR cycle, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each PCR cycle, additional reporter dye molecules are released from the probe, increasing the fluorescence intensity. Fluorescence intensity is recorded at each cycle of the PCR by the Real-Time PCR machine.

The assay consists of labelled probes in one test specific for SARS-CoV-2 (ORF1ab, S and M gene targets) in FAM, Cy5 and ROX channels, respectively; and a second parallel test to detect Influenza A (Flu A), Influenza B (Flu B) and Respiratory Syncytial Virus (RSV A and B) using FAM, Cy5 and ROX fluorophores respectively.

The assay includes an internal extraction control (genesig® Easy RNA Internal Extraction control), which may be added to the IVD nucleic acid extraction system (not provided) to prove efficient RNA extraction, detect PCR inhibition and confirm the integrity of the PCR run.

The internal extraction control assay (which is from a non-biologically relevant exogenous source) is present in both tests and the probe is labelled with the HEX fluorophore.

Positive controls

The kit contains positive control templates for each of the targets, combined into a single tube. This can be run to confirm amplification is being detected in each fluorescence channel. 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 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
ORF1ab/S/M primer/probe mix (BROWN)	220 µl
FluA/FluB/RSV primer/probe mix (BROWN)	220 µl

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

Component - Resuspend in oasig® resuspension buffer	Volume
oasig® Lyophilised OneStep 2X RT-qPCR Mastermix (GOLD)	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
genesig® Winterplex Positive Control Template (RED)*	800 µl

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

5. **Resuspend the internal control template 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
genesig® Winterplex Internal extraction control (IEC) (BLUE)	1000 µl

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. Add 20 µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer.

2. Complete RNA extraction according to the manufacturer's protocols.

Real-Time PCR detection protocol

1. **Prepare 2 reaction mixes, one for each primer/probe mix. Include sufficient reactions for all samples, positive and negative controls.**

ORF1ab/S/M reaction mix:

Component	Volume
Resuspended Onestep Lyophilised Master Mix	10 µl
Resuspended ORF1ab/S/M primer/probe mix	2 µl
Final Volume	**12 µl**

FluA/FluB/RSV reaction mix:

Component	Volume
Resuspended Onestep Lyophilised Master Mix	10 µl
Resuspended FluA/FluB/RSV primer/probe mix	2 µl
Final Volume	**12 µl**

Pipette 12µl of this mix into each well according to your qPCR experimental plate set up.

1. **Prepare RNA templates for each of your samples.**
   Please ensure that the samples are suitable in terms of purity, concentration, and RNA integrity.

2. **Pipette 8 µl of RNA template into each well, according to your experimental plate set up.**
   For negative control wells use 8 µl of RNase/DNAse free water. The final volume in each well is 20 µl.

3. **Pipette 8 µl of the positive control template, according to your experimental plate set up.**
   The final volume in each well is 20 µl

qPCR amplification protocol

The following protocol is recommended for optimum amplification:

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	10 s	95°C
	Annealing and extension (DATA COLLECTION) *	60 s	60°C

* Fluorogenic data should be collected during this step through the **FAM, HEX/VIC, ROX** and **Cy5** channels

Interpretation of results

ORF1ab/S/M reaction mix:

SARS CoV-2 Targets			Internal Extraction Control	Result
ORF1ab FAM (465-510)	S gene CY5 (618-660)	M gene ROX (575-610)	VIC/HEX/Yellow555 (533-580)	
(+)	(+)	(+)	(+) / (-)	SARS-CoV-2 Positive*
(+)	(+)	(-)	(+) / (-)	SARS-CoV-2 Positive*
(+)	(-)	(+)	(+) / (-)	SARS-CoV-2 Positive*
(+)	(-)	(-)	(+) / (-)	SARS-CoV-2 Positive*
(-)	(+)	(-)	(+) / (-)	SARS-CoV-2 Positive*
(-)	(-)	(+)	(+) / (-)	SARS-CoV-2 Positive*
(-)	(-)	(-)	(+)	SARS-CoV-2 Negative
(-)	(-)	(-)	(-)	Result invalid, repeat testing of sample

To ensure PCR run validity, the PCT should produce amplification in the FAM channel for the ORF1ab target, amplification in the Cy5 channel for the S gene target, and amplification in the ROX channel for the M gene target.

FluA/FluB/RSV reaction mix:

Flu A FAM (465-510)	Flu B Cy5 (618-660)	RSV ROX (575-610)	Internal Extraction Control VIC/HEX/Yellow555 (533-580)	Result[!]
(+)	(-)	(-)	(+) / (-)	Flu A Positive*
(-)	(+)	(-)	(+) / (-)	Flu B Positive*
(-)	(-)	(+)	(+) / (-)	RSV Positive*
(-)	(-)	(-)	(+)	Test Negative
(-)	(-)	(-)	(-)	Result invalid, repeat testing of sample

To ensure PCR run validity, the PCT should produce amplification in the FAM channel for the Influenza A target, amplification in the Cy5 channel for the Influenza B target, and amplification in the ROX channel for the RSV target.

*Positive control template is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.