

ProbeSure™ COVID-19 One Step RT-PCR Kit – Duplex + RNase P

Introduction

The ProbeSure™ COVID-19 One Step RT-PCR Kit – Duplex + RNase P is for the qualitative detection of RNA of the novel Coronavirus (SARS-CoV-2) in human respiratory samples. The kit contains assays for two target sequences in the nucleocapsid (N) gene region and an internal RNase P control. The results can be analysed in endpoint or real-time. For full details, please refer to the ProbeSure™ COVID-19 One Step RT-PCR Kit – Duplex + RNase P User Guide at www.3crbio.com.

Included in the kit

- ProbeSure™ one step RT-PCR Master Mix (2x)
- 20x Duplex Assay Mix (Green lid) for N Gene targets N1 (reports with FAM) + N2 (reports with HEX)
- 20x Assay Mix (Orange lid) for RNase P gene (reports with FAM)

Not included in the kit

- PCR plate and optically clear seal
- PCR-grade water
- Template RNA and +/- Controls

Storage

All components must be stored at -20 °C upon arrival. Avoid repeated freeze / thaw cycles.

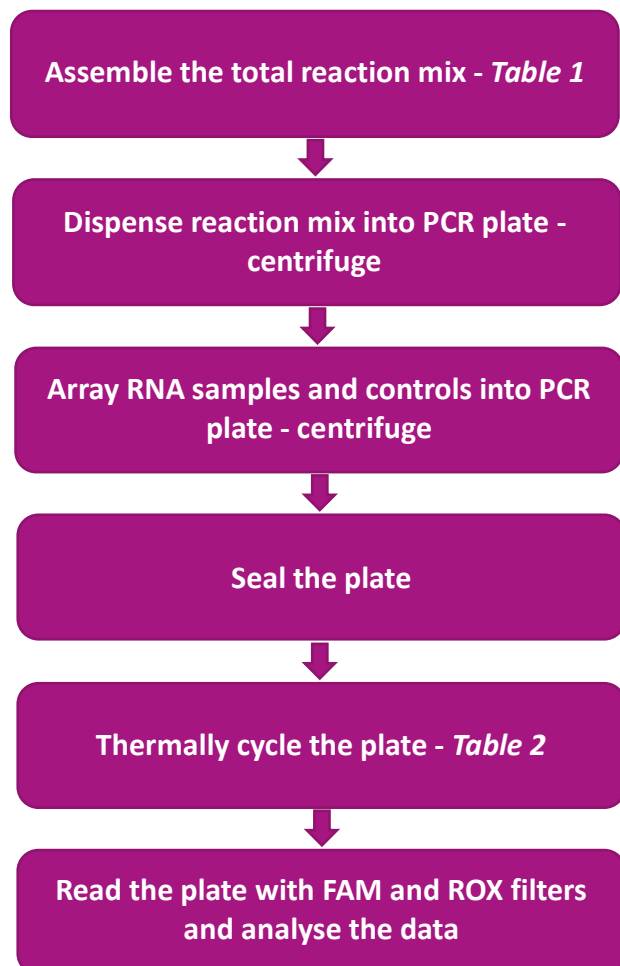
Safety warnings and precautions

This product should only be handled by trained laboratory personnel. Wear suitable PPE when using the product. In case of contact with skin or eyes, wash immediately with water.

	96-well plate	384-well plate
2x ProbeSure-RT Master Mix	10.0	2.5
20X Primer/Probe Mix for N1 + N2 or RNase P ¹	1	0.25
Water	Variable	Variable
RNA ²	Up to 9.0	Up to 2.25
TOTAL	20.0	5.0

Table 1. Reagent volume for assembly of reaction mix. Total volumes indicated are recommended for the associated PCR plate type. Include positive, negative, and no-template controls. ¹ A separate total reaction mix must be assembled for both the N1 + N2 assay and the RNase P assay. ² Add the maximum volume of RNA possible to maximise RNA copy number in the reaction. However, this should be determined experimentally as some crude extraction methods may introduce PCR inhibitors.

ProbeSure™ Covid-19 process flow



Step	Description	Temperature	Time	N°. Cycles
1	Reverse Transcription	50°C	10 min	1
2	Enzyme activation	95°C	10 min	1
3	Template denaturation	95°C	15 secs	50
	Annealing and extension	60°C	60 secs	

Table 2. Thermal cycling conditions for ProbeSure™ COVID-19 one step RT-PCR. PCR can be carried out on any Peltier or water bath thermal cycler.

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Data analysis

After thermal cycling, read the FAM, HEX and ROX fluorescence in a FRET-capable plate reader. Data can be analysed in real-time or endpoint.

If analysing in endpoint, import the data into a genotype cluster analysis software package. Using the Duplex + RNase P kit, the data should be viewed as two separate cluster plots: one for N1 (FAM) and N2 (HEX) and one for RNase P (FAM) control. For a sample to be COVID-19 positive it would need to be positive on the N1 + N2 and RNase P data plots. The data should be assessed as described in *Table 3*.

N1	N2	RNase P	Result
+	+	+	COVID-19 positive
-	+	+	COVID-19 negative
+	-	+	COVID-19 negative
-	-	+	COVID-19 negative
-	-	-	Reaction failed
+	+	-	False positive
+	-	-	False positive
-	+	-	False positive

Table 3. Analysis of the results of the cluster plots for the ProbeSure™ COVID-19 one step RT-PCR kit.

Ordering information

Please visit www.3crbio.com

Licence information

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