

ProbeSure™ COVID-19 One Step RT-PCR Kit – Multiplex

Introduction

The ProbeSure™ COVID-19 One Step RT-PCR Kit – Multiplex 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 – Multiplex User Guide at www.3crbio.com.

Included in the kit

- ProbeSure™ one step RT-PCR Master Mix (2x)
- 10x Multiplex Assay Mix (Purple lid) for N Gene targets N1 (reports with FAM), N2 (reports with HEX) and RNase P control (reports with ATTO 647)

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.

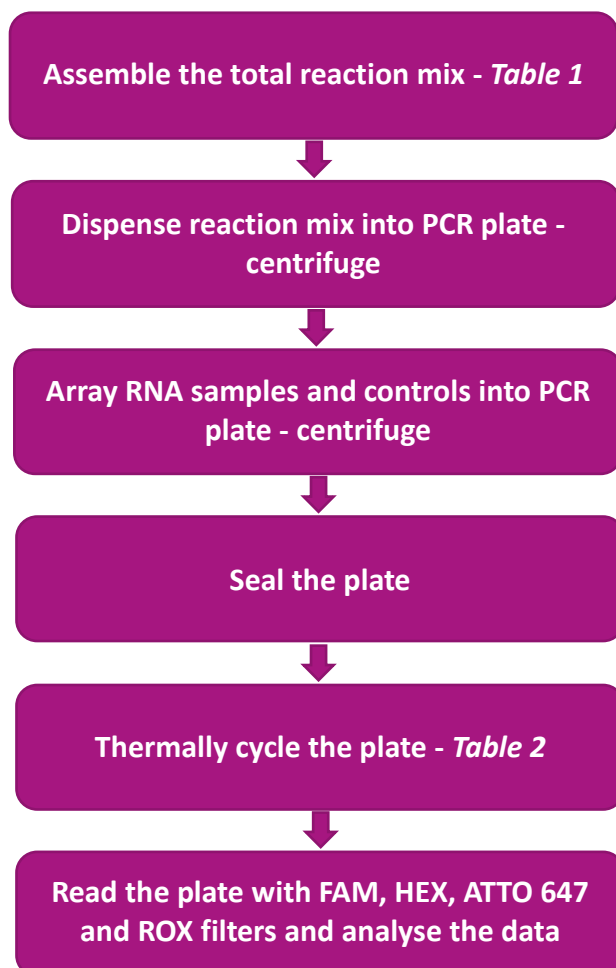
Safety warnings and precautions

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
10X Primer/Probe Mix for N1 + N2 + RNase P	2	0.5
Water	<i>Variable</i>	<i>Variable</i>
RNA ¹	<i>Up to 8.0</i>	<i>Up to 2.0</i>
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 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	45
	Annealing and extension	60°C	30 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

Data analysis should be performed with the software of the real-time PCR instrument according to the manufacturer’s instructions.

Samples should be considered positive for each assay if the cycle threshold (Ct) value is equal to or below 34 for FAM and 35 for HEX. If the Ct value is greater than indicated, the sample should be considered negative. Please note that Ct values might vary with QPCR instrument being used for the testing. These Ct values were validated on ABI 7900.

The data should be assessed as described in *Table 3*.

N1 (FAM)	N2 (HEX)	RNase P (ATTO 647)	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 ProbeSure™ COVID-19 one step RT-PCR kit.

Ordering information

Please visit www.3crbio.com

Licence information

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For any queries about this guide please contact:

Email: support@3crbio.com

Tel: 01279 940 983

www.3crbioscience.com