Quick guide for the preparation of probe and primers for 2019-nCoV ValuPanel reagents

The 2019-nCoV ValuPanel reagents are probes and primers delivered in individual tubes in a dried format. Please follow the steps below to hydrate the individual primers and probes, and subsequently to prepare the primer and probe mixtures for each target. The following protocol is based on probe and primer concentrations as recommended by the CDC for Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes.

Following this process, 1.5 µL of the prepared primer probe mix for a specific target should be used in a 20 µL reaction.

1. Re-suspend the oligonucleotides

Re-suspend the dried probes and primers to achieve individual 100 µM stock solutions for each oligonucleotide. Re-suspension in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8) is recommended. Table 1 details the suspension volume for each of the standard 2019-nCoV ValuPanel reagents supplied by LGC Biosearch Technologies. For example, add 1 mL of TE buffer to a tube containing 100 nmol of oligonucleotide.

Amount of dried oligonucleotide per tube (nmol)	Re-suspension volume to achieve 100 µM stock (mL)
25	0.25
100	1
250	2.5
1000	10

Table 1. Re-suspension of dried oligonucleotide using TE buffer (10 mM Tris, 0.1 µM EDTA) to achieve 100 µM stock.

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2. Prepare probe and primer mixtures for each target

For each target (N1, N2, RNaseP), a combined primer probe mix should be prepared containing a total of 22.5 nmol of oligonucleotides per tube. The proportions of each primer and the probe required in this mix are shown in table 2.

	Primer probe mix (nmol per tube)
Forward primer	10
Reverse primer	10
Probe	2.5
TOTAL	22.5

Table 2. Proportion of each primer and probe required per target.

Using the prepared stock solutions (100 µM) for each oligonucleotide (prepared in step 1), combine the two primers and the probe for each target to create a primer probe mix. We recommend preparing 1.5 mL of primer probe mix as detailed in table 3.

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	Volume of 100 µM stock (µL)	Concentration in prepared 1.5 mL primer probe mix (µM) to 2 d.p	Final concentration in reaction (using 1.5 µL in 20 µL total reaction) (nM)
N1 mix			
2019-nCoV_N1-F	100	6.67	500
2019-nCoV_N1-R	100	6.67	500
2019-nCoV_N1-P	25	1.67	125
Nuclease-free water	1,275	-	
N2 mix			
2019-nCoV_N2-F	100	6.67	500
2019-nCoV_N2-R	100	6.67	500
2019-nCoV_N2-P	25	1.67	125
Nuclease-free water	1,275	-	
RP mix			
RP-F	100	6.67	500
RP-R	100	6.67	500
RP-P	25	1.67	125
Nuclease-free water	1,275	-	

Table 3. Preparation of combined primer probe mixes using individual primer and probe stock solutions for each of the three targets (N1, N2, RP).

Aliquot the prepared primer probe mix in 300 µL volumes into five pre-labelled tubes. Store a single working aliquot at 2-8 °C in the dark. Store the remaining aliquots at -20 °C. Do not re-freeze thawed aliquots.

Please note that this product, the 2019-nCoV ValuPanel Reagents, may not be used under the FDA's EUA for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.