July 2022

SARS-CoV-2 Neo Assay Kit
Instructions for Use (Protocol Sheet)

222115, 222117

QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

R1

The protocol sheet is available electronically and can be found under the resource tab of the product page on **www.qiagen.com**.

Sample to Insight

General information

The SARS-CoV-2 Neo Assay Kit (cat no. 222115 and 222117) is intended for molecular biology application for epidemiological research using RT-PCR.

The SARS-CoV-2 Neo Assay Mix 20x should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer and protected from light.

The SARS-CoV-2 Neo Assay is optimized to work with the QIAprep&™ Viral RNA UM Kit (cat no. 221413, 221415, and 221417), (www.qiagen.com/HB-2830) and SARS-CoV-2 Neo Positive Control (cat. no. 222710) on human samples collected with nasal, nasopharyngeal, or oropharyngeal swabs stored in non-fixation transport media like UTM, VTM, PBS, ESwabs®, Virocult™, or 0.9% NaCl. Alternative starting materials e.g. saliva and gargle are suitable as well.

SARS-CoV-2 Neo targets in the viral genome

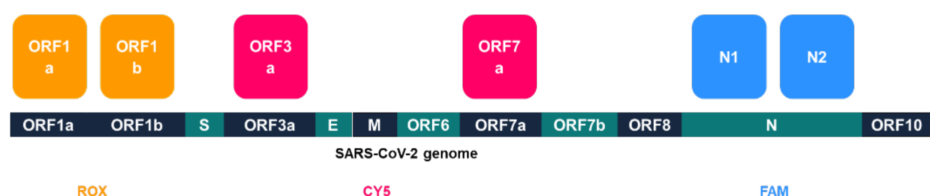

Figure 1. SARS-CoV-2 Neo assay targets in the viral genome.

The SARS-CoV-2 Neo Assay Kit contains primers and probes for detection of N1, N2, ORF1a, ORF1b, ORF3a, and ORF7a within the SARS-CoV-2 viral genome. Each tube of the SARS-CoV-2 Neo Assay Kit contains a mixture of 12 primers and six probes purified by HPLC at a 20x concentration (Table 1). Each tube of the kit contains sufficient volume for 600 x 20 µl reactions.

Table 1. Kit content

Tube	Content	Concentration assay mix	End concentration
SARS-CoV-2 Neo Assay	Primer-Probe mix	20x	1x

Notes before starting

The QIAprep& Viral RNA UM Kit protocol has been optimized to generate best results when combined with the SARS-CoV-2 Neo Assay Kit. Therefore, we recommend to use the reaction conditions specified here. Optionally, the SARS-CoV-2 Neo Positive Control can be added to the experiment set up.

Important:

- The amount of human sampling control assay of the QIAprep& Viral RNA UM kit to be used with the SARS-CoV-2 Neo Assay Kit was optimized.

- Do not add ROX to your QIAprep& Viral RNA UM Kit master mix.*
- Do not add the IC RNA Assay of the QIAprep& Viral RNA UM Kit to your master mix.

Procedure

1. Before use, thaw the QIAprep& Viral RNA UM Prep Buffer, QIAprep& Viral RNA Master Mix, QIAprep& Human Sampling IC Assay, SARS-CoV-2 Neo Assay Mix 20x, SARS-CoV-2 Neo Positive Control (optional), and RNase-Free Water. Vortex the assays, prep buffer, and the master mix. Mix the positive control by inverting the tubes 5X. Spin all tubes down to prevent liquid collection in the lid.

   Note: We recommend to include one well with the positive control for each run by using 8 µl of the positive control instead of the sample.
2. Prepare a reaction mix according to Table 2 and mix thoroughly.

Table 2. Reaction mix setup

Component	1 reaction (20 µl)	Final concentration
Viral RNA Master Mix, 4x	5 µl	1x
SARS-CoV-2 Neo Assay Mix 20x	1 µl	1x
Human Sampling IC Assay, 20x	0.5 µl	0.5x
RNase-free water	Fill up to 10 µl	–
Prepared sample (combined at step 6)	10 µl	–
Total reaction volume	**20 µl**	**–**

Important: Make sure to exclude the reagent kit supplied IC RNA and ROX, as the respective dyes are used for SARS-CoV-2 detection within the Neo Assay mix.

* ROX-dependent instruments can be used without ROX as normalization dye, since the analysis focuses on signal presence/absence but not on relative comparisons.

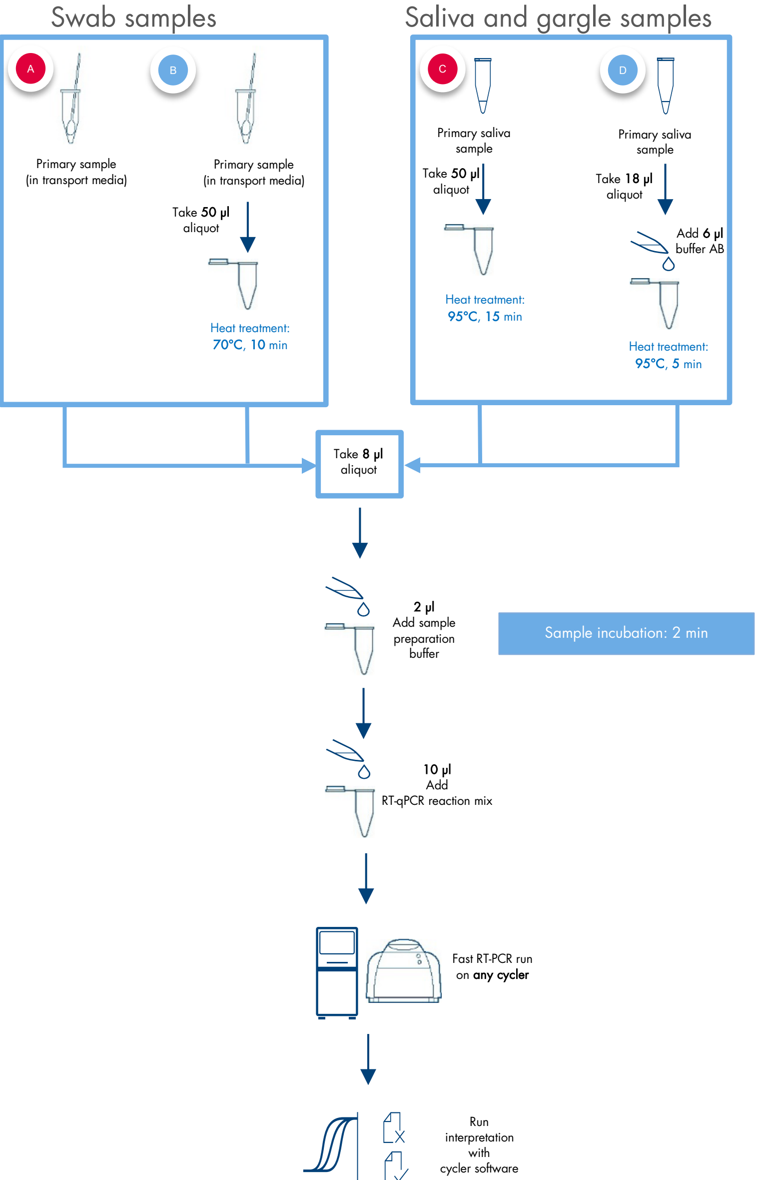

Figure 2. Procedure overview

The next steps differ for different sample types, choose the correct procedure depending on your sample type.

Swab samples (Workflow option A and B)

3. Vortex vigorously the primary sample tube containing the swab in transport media.
4. Optional sample heat treatment (recommended):
   - 4a. Either the entire primary sample or an aliquot of 50 µl can be submitted for heat treatment. Ensure that the complete sample volume is appropriately heated.
   - 4b. Incubate at 70°C for 10 min.
   - 4c. Centrifuge the plate/tube briefly.
   - 4d. Proceed to step 5.

Saliva/gargle samples without QIAprep&™ Buffer AB (Workflow option C)

Heat pre-treatment of samples

3. Vortex saliva or gargle samples vigorously. Either the entire primary sample or an aliquot of 50 µl can be submitted for heat treatment. Ensure that the complete sample volume is appropriately heated, especially for volumes of samples exceeding 100 µl.

   Note: Bigger volumes will require longer incubation times

4. Required heat treatment:

   4a. Incubate at 95°C for 15 min.

   4b. Centrifuge the plate/tube briefly.

   Note: If a saliva sample is very viscous, we recommend a two-step heat treatment. Heat the primary sample to 80°C for 10 min to facilitate pipetting. Subsequently follow up with the regular heating step at 95°C for 15 min in step 4.

   4c. Continue with step 5 below.

Saliva/gargle samples with QIAprep& Buffer AB (Workflow option D)

Heat pre-treatment of samples with QIAprep& Buffer AB

3. Before use, prepare the QIAprep& Buffer AB according to Table 3 and mix thoroughly.

Table 3. QIAprep& Buffer AB setup

Component	1 rxn	Final concentration
QIAprep& Buffer A	3.75 µl	1x
QIAprep& Buffer B**	2.25 µl	1x
Total reaction volume	6 µl	–

   3a. Pre-dispense 6 µl of Buffer AB into a PCR tube or the well of a PCR plate.

   3b. Vortex saliva or gargle samples vigorously. Add 18 µl of sample to the tube or well containing the Buffer AB. Mix by pipetting up and down at least twice.

   Note: If a saliva sample is very viscous, we recommend a two-step heat treatment. Heat the primary sample to 80°C for 10 min to facilitate pipetting. Subsequently follow up with the regular heating step at 95°C for 5 min in step 4.

4. Seal the tube/plate and incubate for 5 min at 95°C. Proceed to step 5.

Next steps common to all sample types:

5. Dispense 2 µl of Viral RNA UM Prep Buffer into each PCR tube or a well of a PCR plate.
6. Gently mix by pipetting up and down the (pre-treated) sample and transfer 8 µl of the sample (or the positive control) to the same PCR tube or wells containing the Viral RNA UM Prep Buffer. Mix by pipetting up and down at least twice. Incubate at room temperature for 2 min.

   Note: Incubation time starts after adding the last sample to the Viral RNA UM Prep Buffer.

7. Add 10 µl of the reaction mix prepared in step 2.

** contains Proteinase K

8. Consider these important steps:
   8a. Seal the plate/tube thoroughly to prevent cross-contamination. In case an adhesive film is used, make sure to apply pressure uniformly across the entire plate to obtain a tight seal across individual wells.
   8b. Vortex gently for 10–30 s with medium pressure. Place the plate in different positions while vortexing to ensure an equal contact with the vortex platform.
   8c. Centrifuge the plate/tube briefly to collect liquid at the bottom of the plate/tube.
9. Immediately proceed to step 10.

   Note: The complete reaction can be stored only when using heat-treated samples up to 1 h at room temperature or for a longer period, frozen at –30 to –15°C.
10. Place the tubes or plates in the real-time cycler and perform cycling according to below conditions Table 4.
11. Program the real-time cycler before reaction setup according to Table 4.

    Note: Data acquisition should be performed during the annealing/extension step.

Table 4. Cycling conditions

Step	Time	Temperature	Ramp rate
RT-step	10 min	50°C	Maximal/fast mode
PCR initial heat activation	2 min	95°C	Maximal/fast mode
2-step cycling (40 cycles)			
Denaturation	5 s	95°C	Maximal/fast mode
Combined annealing/extension	30 s	58°C	Maximal/fast mode

12. For possible result interpretation, refer to Table 5.

    Note: If the positive control is included in the run, a signal below Ct 40 is expected.

Table 5. Potential result interpretation

SARS-CoV-2 RNA signal in at least one channel: FAM/ROX/CY5	Sampling control	Status	Result
+	+	VALID	Positive
+	–	VALID	Positive
–	+	VALID	Negative, virus not detected
–	–	Inconclusive	Repeat test using a new sample

The positive control can be used for run validity.

Symbols

The following symbols appear in this document.

Symbol	Symbol definition
REF	Catalog number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Manufacturer

Revision history

Revision	Description
R1, July 2022	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN® kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Trademarks: QIAGEN®, Sample to Insight®, QIAprep&™ (QIAGEN Group); Eswabs®, Virocult™. Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.
1127966 07/2022 HB-3101-001 © 2022 QIAGEN, all rights reserved.

Ordering **www.qiagen.com/shop** | Technical Support **support.qiagen.com** | Website **www.qiagen.com**