January 2022

Quick-Start Protocol

QIAprep&amp Buffer AB

The QIAprep&amp Buffer AB (cat. nos. 221513, 221515, and 221517) should be stored upon receipt at –30 to –15°C in a constant-temperature fridge and protected from light.

Further information

- *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The QIAprep&amp Buffer AB is intended for increasing sensitivity of the QIAprep&amp Viral RNA workflow when using sample types such as neat/pure saliva and gargle samples or other sample types requiring pre-treatment.
- The QIAprep&amp Buffer AB is intended to be used in conjunction with a QIAprep&amp Viral RNA UM Kit (cat. nos. 221413, 221415, or 221417). Refer to these kits' handbook for safe and proper use.
- For samples such as nasal, nasopharyngeal, or oropharyngeal swabs that are stored in non-fixation transport media such as UTM, VTM, PBS, ESwabs®, Viroculti™, or 0.9% NaCl, the use of the QIAprep&amp Buffer AB is not required; instead, refer to the *QIAprep&amp Viral RNA UM Kit Handbook*.
- The protocol in this Quick-Start Protocol includes a heat treatment step before the sample preparation step. QIAGEN cannot guarantee that this heat treatment step will inactivate 100% of viral particles. The inactivation of virus needs to be verified and validated by users.

Kit contents

QIAprep&amp Buffer AB	0.7 ml	4.1 ml	16.4 ml
Catalog no.	221513	221515	221517
QIAprep&amp Buffer A	0.45 ml	2x 1.3 ml	8x 1.3 ml
QIAprep&amp Buffer B	0.25 ml	1.5 ml	4x 1.5 ml
Quick-Start Protocol	1	1	1

Procedure

Heat pre-treatment of samples with the QIAprep&amp Buffer AB

1. Before use, prepare the QIAprep&amp Buffer AB according to Table 1 and mix thoroughly.
2. Pre-dispense 6 µl of Buffer AB into a PCR tube or the well of a PCR plate
3. Add 18 µl of sample to the tube or well containing the Buffer AB. Mix by pipetting up and down at least twice.

   Note: Saliva samples can have high viscosity. Heating the primary sample to 80°C for 10 min can lower viscosity of the sample and facilitate pipetting of saliva samples. Heating the primary sample to 80°C for 10 min does not replace the heating step at 95°C for 5 min in step 4.
4. Seal the tube/plate and incubate for 5 min at 95°C.
5. Briefly centrifuge the tubes. Gently mix by pipetting up and down at least twice, and transfer 8 µl of heat pre-treated sample into new PCR tube or well and proceed to step 6 below.

Table 1. Saliva Prep Buffer setup

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

* contains Proteinase K

PCR Setup

6. Prepare a reaction mix according to Table 2 and mix thoroughly.
7. Add 12 µl of the reaction mix prepared in step 6 to 8 µl of sample prepared in step 5. Mix by pipetting up and down at least twice. The complete reaction can be stored up to 1 h at room temperature or stored frozen at –30 to –15°C for the longer period.

Table 2. Reaction mix setup

Component	96/384-well block	Final concentration
Viral RNA Master Mix, 4x*	5 µl	1x
20x primer–probe mix	1 µl	1x
RNA IC Template + Assay, 10x*	2 µl	1x
Human Sampling IC Assay, 20x*	1 µl	1x
ROX Reference Dye (ABI instruments only)*	1 µl/0.1 µl†	1x
Viral RNA UM Prep Buffer*	2 µl	1x
RNase-Free Water*	Fill up to 12 µl	-
Prepared sample (after step 5)	8 µl	-
Total reaction volume	**20 µl**	**–**

* Kit component of QIAprep&amp Viral RNA UM Kit (cat. nos. 221413, 221414, 221415, and 221417). Refer to the *QIAprep&amp Viral RNA UM Handbook* for safe and proper use.

† To be used as a 20x concentrate for high-ROX dye cyclers (i.e., ABI PRISM® 7000, Applied Biosystems® 7300, 7900, and StepOne® Real-Time PCR Systems) and as a 200x concentrate for low ROX-dye cyclers (i.e., Applied Biosystems 7500, ViiA™ 7, and QuantStudio® Real-Time PCR Systems).

8. **Important consideration**:

   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.

   Mix gently by vortexing for 10–30 s with medium pressure. Place the plate in different positions while vortexing, to ensure an equal contact with the vortex platform.

   Centrifuge the plate/tube briefly to collect liquid at the bottom of the plate/tube.

9. Program the real-time cycler according to Table 3.

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

Table 3. 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

* Annealing temperatures can be adapted between 55–62°C depending on primer/probe set used. For further details on cycling conditions, primer/probe concentrations, and annealing temperature, visit the product page (**www.qiagen.com/qiaprepandamp-resources**).

10. Place the tubes or plates in the real-time cycler and start the cycling program.
11. For results interpretation, refer to the table "Possible outcome" in the *QIAprep&amp Viral RNA UM Kit Handbook*: **www.qiagen.com/HB-2830**.

Document Revision History

Date	Changes
04/2021	Initial release
05/2021	Revised the volume of both QIAprep&amp Buffers A and B in the Kit Contents table.
01/2022	Added the catalog number for the new kit size (100 rxn; cat. no. 221413)

Scan QR code for the product page and supplementary protocols.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.