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Product Information

Viral RNA Extraction Buffer

Catalog Number **VRE100**
Storage Temperature -20 °C

Product Description

Viral RNA Extraction Buffer is a 4x working solution that is optimized to provide rapid, room-temperature lysis of enveloped RNA viruses, including SARS-CoV-2, for direct nucleic acid detection by molecular assays. Viral RNA Extraction Buffer is mixed 1:3 with virus-containing samples and incubated for 5 minutes at room temperature, resulting in stabilized, exposed RNA.

If detecting RNA via qRT-PCR, lysis can be performed in a qPCR plate or strips. qRT-PCR master mix containing primers and probe is added directly after the 5-minute lysis. There is no RNA cleanup step required between lysis and detection.

This product is compatible with enveloped viral particles in saliva, saline, Amies medium, and viral transport medium. If detecting viral particles in saliva, removal of precipitates via centrifugation prior to lysis is highly recommended for optimal sensitivity.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Heating of samples prior to or during lysis with Viral RNA Extraction Buffer is not recommended as high temperatures, even for brief periods, can rapidly degrade viral RNA.

Follow all appropriate biosafety procedures when handling samples potentially containing active virus.

Storage/Stability

Viral RNA Extraction Buffer should be stored at -20 °C. Thawed material kept on ice can be aliquoted and re-frozen up to two times.

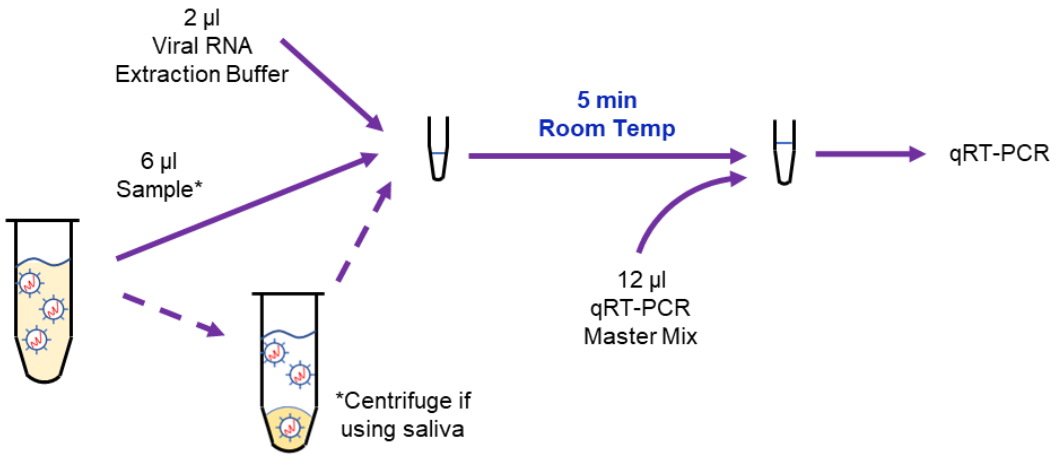

Figure 1. Workflow schematic for RNA extraction and stabilization using Viral RNA Extraction Buffer and downstream analysis via qRT-PCR.

Sample SARS-CoV-2 detection qRT-PCR Workflow

Materials

- Viral RNA Extraction Buffer (Sigma-Aldrich catalog VRE100)
- One-Step Quantitative RT-PCR ReadyMix™ (Sigma-Aldrich catalog QR0200)
- Sample containing enveloped RNA virus
- Target-specific primers
- Target-specific fluorescent probe
- Plastic PCR tubes or plates recommended by the PCR instrument manufacturer
- Real-time PCR instrument

Viral Lysis

- Thaw assay reagents on ice:
  - Viral RNA Extraction Buffer
  - 2x qRT-PCR Ready Mix (QR0200 component)
  - $MgCl_2$ (QR0200 component)
  - Primer and probe aliquots if frozen
- If working with saliva, centrifuge at least 100 µl of sample for 5 minutes at >13,000 x *g*.
- Transfer 6 µl of saline or saliva sample directly to PCR tube.
- Add 2 µl of Viral RNA Extraction Buffer and mix by pipetting up and down at least 3 times.
- Incubate for 5 minutes at room temperature.

qRT-PCR preparation

During lysis, assemble a master mix for the appropriate number of reactions. Shown here are volumes of reagents used in each reaction:

Reagent	**Final Concentration**	**Per 20 µL Reaction**
2x qRT-PCR ReadyMix	1x	10 µL
25 mM $MgCl_2$ (as supplement to 3 mM $MgCl_2$ in 2x ReadyMix)	1.5 mM	1.2 µL
M-MLV Reverse Transcriptase	1 U/µL	0.1 µL
Forward Primer (100 µM)	500 nM (optimizable)	0.1 µL
Reverse Primer (100 µM)	500 nM (optimizable)	0.1 µL
Probe (100 µM)	125 nM (optimizable)	0.025 µL
Nuclease-Free Water		Fill to 12 µL (allowing for 8 µL sample)

After viral lysis is complete, add 12 µL assembled master mix to each 8 µL extraction sample and seal PCR tube or plate. Centrifuge briefly to ensure the liquid is at the bottom of each well.

Note: qRT-PCR master mix cannot be combined with Viral RNA Extraction Buffer before viral lysis. The RNA extraction step must be completed before addition of qRT-PCR reagents.

Program thermal cycler for 100-600 bp fragments and run with appropriate probe detection.

Reverse Transcription		44 °C	30 min
Initial denaturation		94 °C	2 min
45 cycles	Denaturation	94 °C	15 sec
	Annealing, extension, and detection	60 °C, or 5 °C below lowest primer T_m	1 min

Note: Do **not** remove tube lid or plate seal after thermal cycling is complete. Opening samples after amplification causes risk of carryover DNA contamination.

Related products

- Quantitative RT-PCR ReadyMix™ (Catalog Number QR0200)

References

1. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes, *Centers for Disease Control*, https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html
2. Lovatt, A., et al. Validation of Quantitative PCR Assays, *BioPharm*, March 2002, p. 22-32.

Performance Characteristics

Sensitivity

Viral RNA Extraction Buffer consistently detects 1.5x10^3 particles per mL in saline and 5x10^4 particles per mL in saliva of crosslink-inactivated SARS-CoV-2, using 6 µl of sample in a 20 µl qRT-PCR reaction.

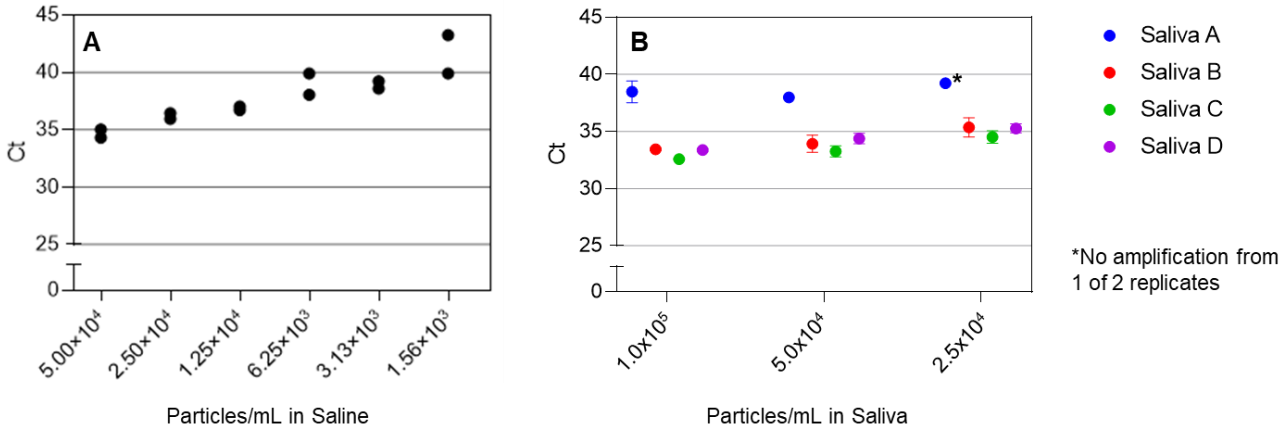

Figure 2. To determine sensitivity of Viral RNA Extraction Buffer, ZeptoMetrix® NATtrol™ crosslink-inactivated SARS-CoV-2 particles at various concentrations were spiked into saline or saliva from 4 healthy individuals. Saliva samples were centrifuged and supernatant was used for testing. 2 µl Viral RNA Extraction Buffer was added to 6 µl sample and incubated for 5 minutes at room temperature for lysis. 12 µl qRT-PCR Master Mix (Sigma-Aldrich QR0200) containing CDC-recommended N2 primers and probe was added directly to the RNA-extracted sample and analyzed on a BioRad CFX Connect™ instrument. **A**, C(t) values obtained with decreasing amounts of viral particles in saline. **B**, C(t) values obtained with decreasing amounts of viral particles spiked into human saliva. Each color represents saliva from a different healthy individual.

Performance Relative to Similar Approaches

Faster results, more robust signal, and more reproducible C(t) values are observed when using Viral RNA Extraction Buffer with saliva compared to another leading method, lysis by Proteinase K and heating.

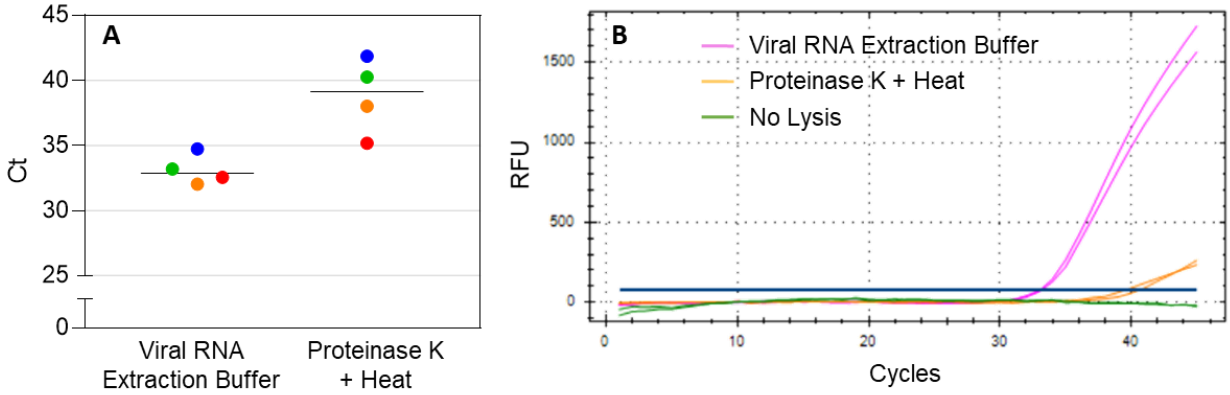

Figure 3. Saliva from 4 healthy individuals was spiked with ZeptoMetrix® NATtrol™ crosslink-inactivated SARS-CoV-2 particles at 1x10^5 viral particles per mL. Saliva with spiked viral particles was either: centrifuged and treated with Viral RNA Extraction Buffer for 5 min; treated with Proteinase K, vortexed for 1 min, heated at 95°C for 5 min, and briefly centrifuged; or left untreated. 8 µl total from each treatment was added to 12 µl RT-qPCR Master Mix (Sigma-Aldrich QR0200) containing CDC-recommended N2 primers and probe and analyzed on a BioRad CFX Connect™ instrument. **A**, C(t) values from four different saliva samples. Each color represents saliva from a different individual containing spiked viral particles **B**, Amplification curves from one saliva sample (each treatment analyzed in duplicate).

qRT-PCR Compatibility

Viral RNA Extraction Buffer is compatible with most nucleic acid amplification methods and does not decrease assay sensitivity or reduce fluorescence signal when used at working concentration in qRT-PCR. Compatibility has been verified with the following kits:

- Quantitative RT-PCR ReadyMix™ (Sigma-Aldrich, catalog QR0200)
- KAPA PROBE® FAST One-Step Universal (Sigma-Aldrich, catalog KK4752)
- KiCqStart® One-Step Probe RT-qPCR ReadyMix™ (Sigma-Aldrich, catalog KCQS07)
- TaqPath™ 1-Step Multiplex Master Mix (Thermo Fisher, catalog A28525)

Viral RNA Extraction Buffer is compatible with multiple fluors and has been demonstrated to function without background signal or interference in a 4-fluor multiplex setting using Cy5, Texas Red, FAM, and HEX.

LAMP Compatibility

Viral RNA can be detected in human saliva when Viral RNA Extraction Buffer is paired with RNA detection via colorimetric loop-mediated isothermal amplification (LAMP). Viral RNA Extraction Buffer by itself does not interfere with LAMP.

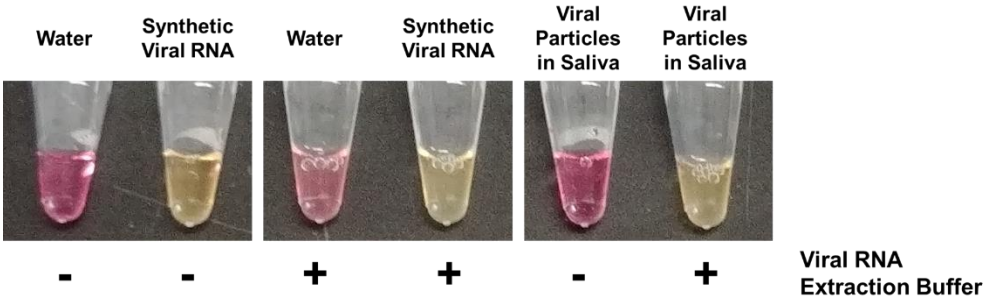

Figure 4. Healthy human saliva was spiked with ZeptoMetrix® NATtrol™ crosslink-inactivated SARS-CoV-2 at $5x10^4$ viral particles per mL, then treated with Viral RNA Extraction Buffer. Viral RNA was detected with a SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB). Pink color indicated a negative result, while yellow indicated a positive result in which SARS-CoV-2 RNA was detected. Synthetic SARS-CoV-2 RNA ($1x10^5$ copies, Twist Bioscience) was used as a positive assay control.

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