

Validated Inactivation Methods for SARS-CoV-2 and COVID-19 positive samples

Internally validated methods for listed samples:

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Whole blood

Heat inactivation at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

Serum/plasma

Heat inactivation at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

Paraformaldehyde (4% final concentration) • incubate at room temperature • 15 min •

Trizol Reagent (900µL Trizol per 100µL sample) • incubate at room temperature • 10 min •

Trizol LS Reagent (3 parts Trizol LS reagent / 1 part sample) • incubate at room temperature
• for 10 min •

Triton X-100 (4% final concentration) • incubate at room temperature • 5 min •
(validated by Dr. Sara Cherry)

Ineffective inactivation

Triton X-100 (1% final concentration) • incubation at room temp or 37 °C • 15 min •
(validated by Dr. Susan Weiss' lab)

Inactivation being evaluated

UV inactivation
(testing by Dr. Sara Cherry)

Urine

Heat inactivation at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

Saliva

Heat inactivation at 95 °C • incubate for 5 min •
(validated by Dr. Sara Cherry)

Cell monolayers

Glutaraldehyde solution (2.5% final concentration) • incubate at room temperature • 15 min •

Paraformaldehyde solution (4% final concentration) • incubate at room temperature • 30 min •
(validated for MERS coronavirus)

Trizol Reagent with 300-400 µL Trizol per 1×10^5 - 1×10^7 cells • incubate at room temperature • 10 minutes • sample volume **must not exceed 10%** of the volume of Trizol Reagent used for lysis

Inactivation being evaluated

Qiagen RNease Mini Plus: RLT-plus lysis buffer

NP-40 lysis buffer (containing 1% NP-40, 2mM EDTA, 10% glycerol, 150mM NaCl, 50mM Tris pH 8.0, and Roche protease inhibitors)

Tissue Samples

Trizol Reagent, 1 mL per 50-100 mg tissue for homogenization in closed system; sample volume must not exceed 10% of the volume of Trizol Reagent used for lysis

Oropharyngeal (OP)/Nasopharyngeal (NP) swabs in Viral Transport Medium (VTM)

Heat inactivation at 56 °C • incubate for 1 hour •
(validated by Dr. Sara Cherry)

Endotracheal Aspirate

Trizol Reagent (900µL Trizol per 100µL sample) • incubate at room temperature • 10 min •

Trizol LS Reagent (3 parts Trizol LS reagent and 1 part sample) • incubate at room temperature • 10 min •

Other Reagents

COPAN eNAT medium

(validated by Dr. Susan Weiss' lab)

Inactivation being evaluated

Qiagen CD1 solution

(testing by Dr. Susan Weiss' lab)

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Documented methods: CDC-approved RNA extraction buffers

(FDA Emergency Use Authorization: <https://www.fda.gov/media/134922/download>)

Must be used following product manual and manufacturer's recommendations.

RNA Extraction Options

For each of the kits listed below, CDC has confirmed that the external lysis buffer is effective for inactivation of SARS-CoV-2.

Instrument/Manufacturer	Extraction Kit	Catalog No.
QIAGEN	² QIAamp DSP Viral RNA Mini Kit	50 extractions (61904)
	² QIAamp Viral RNA Mini Kit	50 extractions (52904) 250 extractions (52906)
QIAGEN EZ1 Advanced XL	² EZ1 DSP Virus Kit	48 extractions (62724) Buffer AVL (19073 or 19089) EZ1 Advanced XL DSP Virus Card (9018703)
	² EZ1 Virus Mini Kit v2.0	48 extractions (955134) Buffer AVL (19073 or 19089) EZ1 Advanced XL Virus Card v2.0 (9018708)
Roche MagNA Pure 24	² MagNA Pure 24 Total NA Isolation Kit	96 extractions (07 658 036 001) External Lysis Buffer (06 374 913 001, 12 239 469 103, 03 246 779 001 or 03 246 752 001)
Roche MagNA Pure 96	² DNA and Viral NA Small Volume Kit	576 extractions (06 543 588 001) External Lysis Buffer (06 374 913 001, 12 239 469 103, 03 246 779 001 or 03 246 752 001)
¹ Roche MagNA Pure LC	² Total Nucleic Acid Kit	192 extractions (03 038 505 001)
¹ Roche MagNA Pure Compact	² Nucleic Acid Isolation Kit I	32 extractions (03 730 964 001)
¹ QIAGEN QIAcube	² QIAamp DSP Viral RNA Mini Kit	50 extractions (61904)
	² QIAamp Viral RNA Mini Kit	50 extractions (52904) 250 extractions (52906)
^{1,3} bioMérieux NucliSENS [®] easyMAG [®] and ^{1,3} bioMérieux EMAG [®] (Automated magnetic extraction reagents sold separately. Both instruments use the same reagents and disposables, with the exception of tips.)		EasyMAG [®] Magnetic Silica (280133) EasyMAG [®] Lysis Buffer (280134) EasyMAG [®] Lysis Buffer, 2 mL (200292) EasyMAG [®] Wash Buffers 1,2, and 3 (280130, 280131, 280132) EasyMAG [®] Disposables (280135) Biohit Pipette Tips (easyMAG [®] only) (280146) EMAG [®] 1000µL Tips (418922)

¹Equivalence and performance of these extraction platforms for extraction of viral RNA were demonstrated with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (K190302). Performance characteristics of these extraction platforms with 2019-nCoV (SARS CoV-2) have not been demonstrated.

²CDC has confirmed that the external lysis buffer used with this extraction method is effective for inactivation of SARS-CoV-2.

³CDC has compared the concentration of inactivating agent in the lysis buffer used with this extraction method and has determined the concentration to be within the range of concentrations found effective in inactivation of SARS-CoV-2.