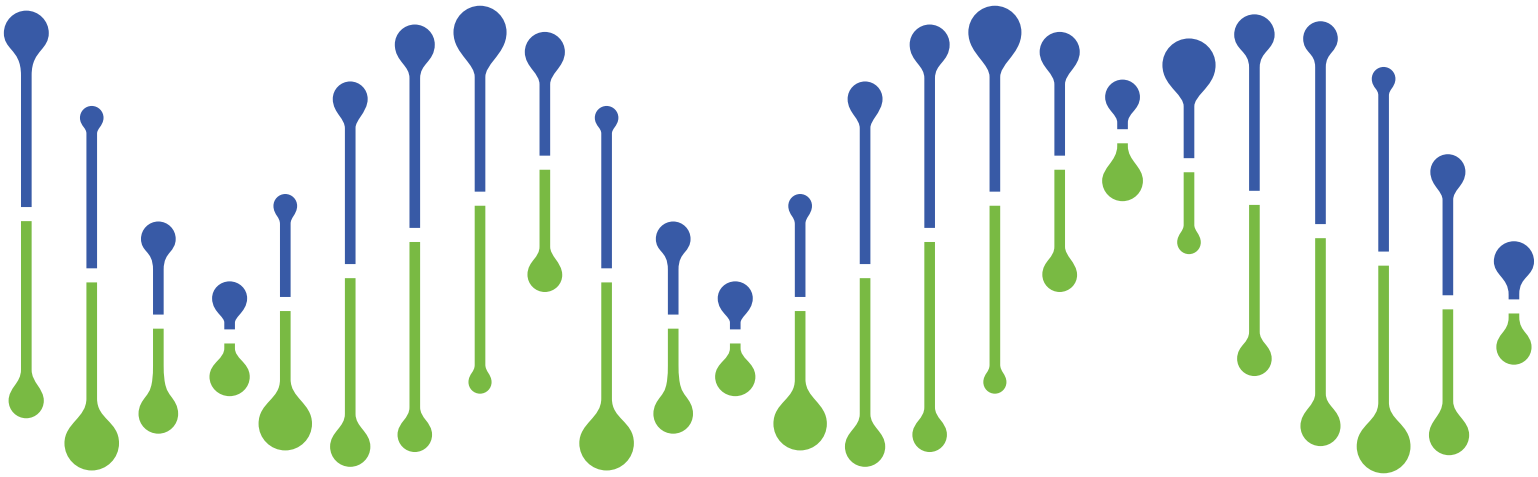
Nonacus Protocol Guide v1.0.3

VirPath - Bead Xtract Viral RNA/DNA Extraction Kit



NONACUS
Advancing Non-Invasive Healthcare

Intended use

The VirPath Xtract Viral DNA/RNA Extraction Kit is intended to be used for rapid total nucleic acid extraction from nasopharyngeal swabs and other biological fluids. The kit has been specifically validated for the extraction of viral RNA from specimens containing the SARS-CoV-2 virus.

Kit contents

Reagent	Volume (1,000 rxns)	Volume (10,000 rxns)	Storage
VirPath Lysis Buffer	1 x 100 ml	4 x 250 ml	Room Temperature
VirPath Target Pure Beads	1 x 40 ml	2 x 200 ml	4°C

Required equipment

- Class II / III Biological safety cabinet
- Single and/or multichannel pipettes (100, 200, 1000 µl)
- PCR-clean filtered tips
- 96-well deep well plates that can accommodate 1 or 2 ml volume per well
- Magnetic separation rack capable of accommodating 96-well deep well plates (Alpaqua #A000380) or equivalent

Additional user supplied consumables

- 100% isopropanol, molecular biology grade
- 100% ethanol, molecular biology grade
- Buffer EB or equivalent buffer saline solution (10 mM Tris-HCl, ph8.0)
- Water, nuclease free molecular biology grade

Storage and handling

Upon receipt, store VirPath Target Pure beads refrigerated at 4°C (do not freeze). VirPath Lysis Buffer is stable at room temperature for 12 months.

Pre-processing of biological specimens

Upon receipt, biological specimens should be handled with caution and only following an onsite risk assessment to ensure safe handling.

Swab in viral transport medium

1. Vortex the tubes containing the swab at maximum speed for 1 minute.
2. Aliquot 200 µl of supernatant and transfer to a fresh 1.5ml tube or 96 deep well plate.

Extraction procedure

Before beginning, remove the VirPath Target Pure beads from storage and place at room temperature for 30 minutes. Prepare a fresh 80% ethanol solution (800 µl per sample are required).

1. Aliquot 200 µl of sample into a fresh 1.5 ml tube or well of a 96 deep well plate.
2. Add 100 µl of VirPath Lysis Buffer to each sample.
3. Vortex the tube(s) / plate for 10 seconds and incubate at room temperature for 10 minutes.
4. Add 40 µl of thoroughly vortexed room-temperature equilibrated VirPath Target Pure beads to each sample.
5. Add 270 µl of 100% molecular grade isopropanol to each sample and mix gently by pipetting up and down 10 times, taking care to avoid the formation of bubbles.
6. Place the tubes / 96 deep well plate on the magnetic stand for 10 minutes at room temperature to pellet the beads on the side of the tubes/wells.
7. Keeping the tubes / 96 deep well plate on the magnetic stand, slowly remove and discard the supernatant, taking care not to disturb the pelleted beads.
8. Add 400 µl of 100% molecular grade isopropanol to each tube/well and incubate at room temperature for 30 seconds.
9. Keeping the tubes / 96 deep well plate on the magnetic stand, slowly remove and discard the supernatant, taking care not to disturb the pelleted beads.
10. Add 400 µl of 80% ethanol to each tube/well and incubate at room temperature for 30 seconds.
11. Repeat steps 9-11 for a total of two 80% ethanol washes.
12. Keeping the tubes / 96 deep well plate on the magnetic stand, slowly remove and discard the supernatant, taking care not to disturb the pelleted beads.
13. Use a 10 µl multichannel or single channel pipette to remove any residual liquid from the tubes/wells.
14. Keeping the tubes / 96 deep well plate on the magnetic stand, incubate at room temperature with open lids for 3-5 minutes or until the beads are dry.
    NOTE: it is important to avoid over-drying of beads, as this can result in a significant loss of RNA/DNA recovered.
15. Remove the tubes / 96 deep well plate from the magnetic stand and resuspend the dried beads in 52 µl of Buffer EB or equivalent buffer saline solution (10 mM Tris-HCl, ph8.0) by pipetting up and down 10-15 times, taking care to avoid the formation of bubbles.
16. Incubate the tubes / 96 deep well plate for 2 minutes at room temperature.
17. Place the tubes / 96 well plate on the magnetic stand for 3-5 minutes at room temperature to pellet the beads on the side of the tubes/wells.
    NOTE: if after 5 minutes the beads have not entirely pelleted to the side of the tube, add 10 µl of Buffer EB or equivalent and wait 3 more minutes for beads to pellet. Keep incrementing the amount of Buffer EB or equivalent added if beads are still not pelleting to the side of the tube until the solution is entirely clear of beads.
18. Carefully recover 50 µl of supernatant and transfer it to a fresh 1.5 ml low-bind tube or 96 well plate.

Limit of Detection (LOD) testing

Synthetic SARS-CoV-2 RNA: ORF, E, N (ATCC® VR-3276SD™) was spiked into negative biofluid samples collected in stabilisation tubes at viral load concentrations equivalent to 100,000 copies / ml to 200 copies / ml. The samples containing the RNA control spike in were subsequently extracted using VirPath Xtract kit, eluted in 50ul of elution buffer and 5ul of elution processed according to VirPath qRT-PCR assay protocol using primers / probes specific for SARS-CoV-2.

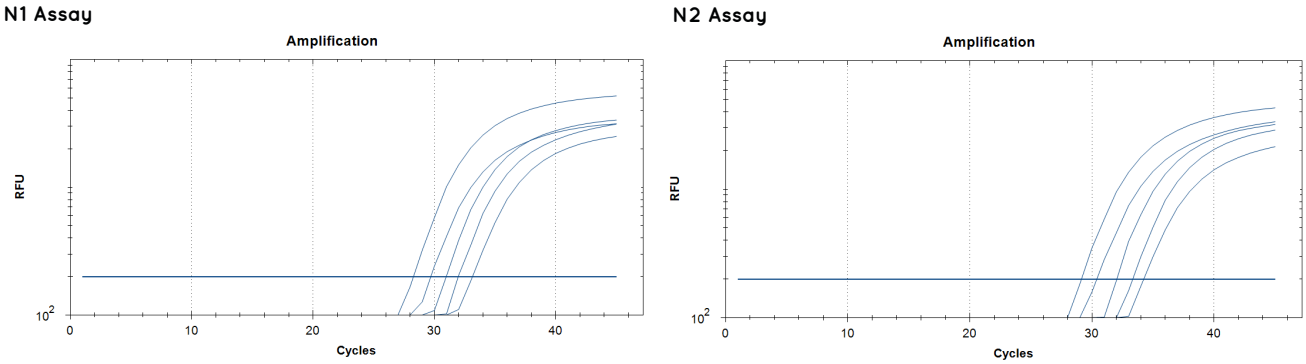


Figure 1: qRT-PCR data of samples containing 100k, 50k, 20k, 10k and 5k copies / ml of Synthetic SARS-CoV-2 RNA control extracted using VirPath Xtract, processed using VirPath qRT-PCR and run on a BioRad CFX96 qPCR instrument.

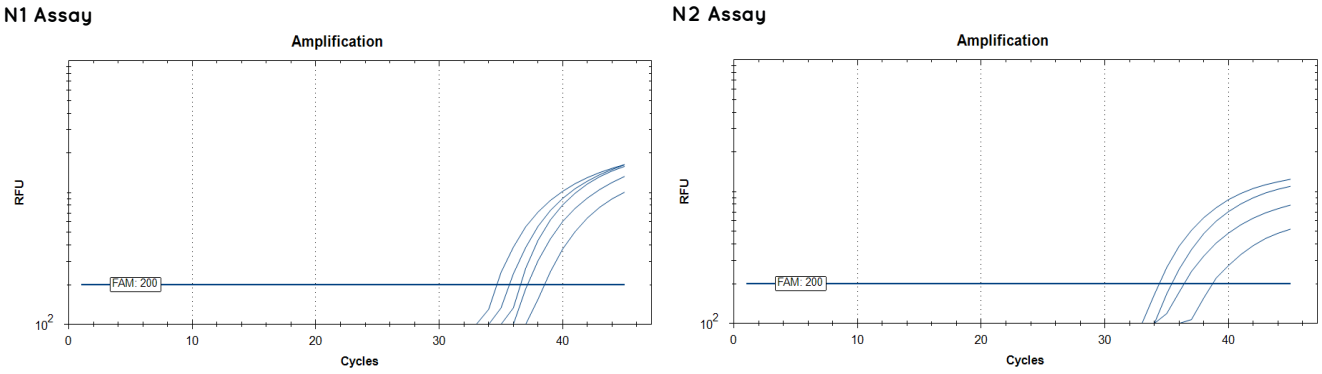
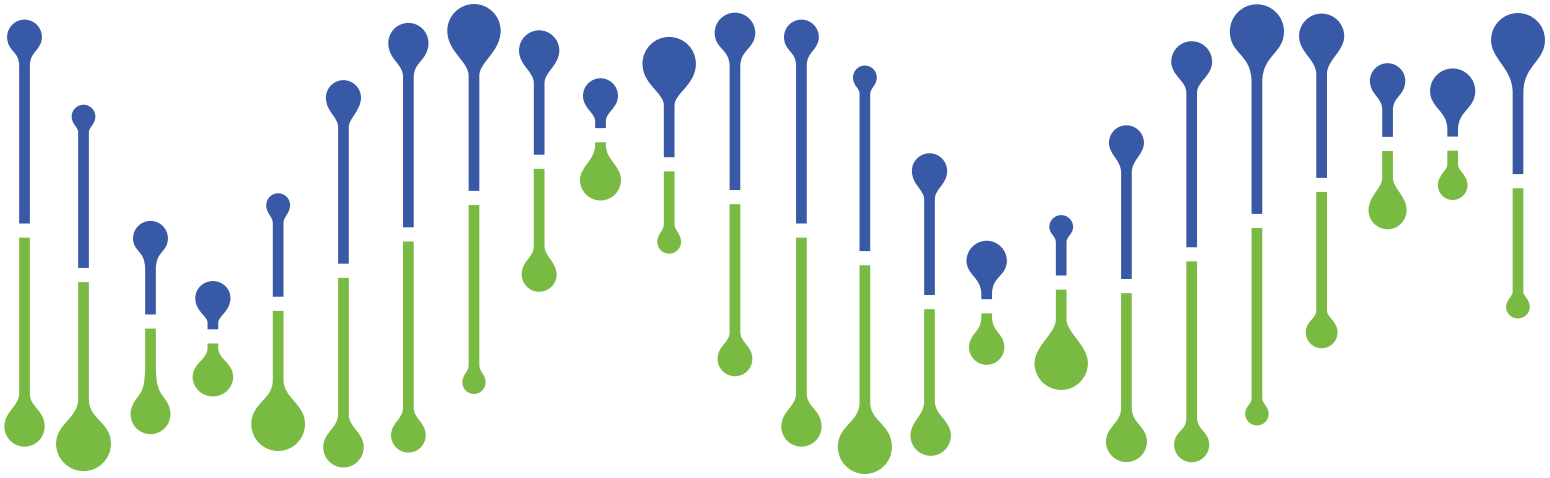


Figure 2: qRT-PCR data of samples containing 2,500, 1000, 500, 200 (N1 assay only) copies / ml of Synthetic SARS-CoV-2 RNA control extracted using VirPath Xtract, processed using VirPath qRT-PCR and run on a BioRad CFX96 qPCR instrument.

Copies / ml	Type	Mean N1 Cq	Mean N2 Cq
100,000	Synthetic RNA	28.36	29.13
50,000	Synthetic RNA	29.65	30.38
20,000	Synthetic RNA	30.76	31.98
10,000	Synthetic RNA	32.01	33.07
5,000	Synthetic RNA	33.24	33.94
2500	Synthetic RNA	35.26	35.20
1000	Synthetic RNA	35.80	36.09
500	Synthetic RNA	37.04	38.92
200	Synthetic RNA	38.57	NaN



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