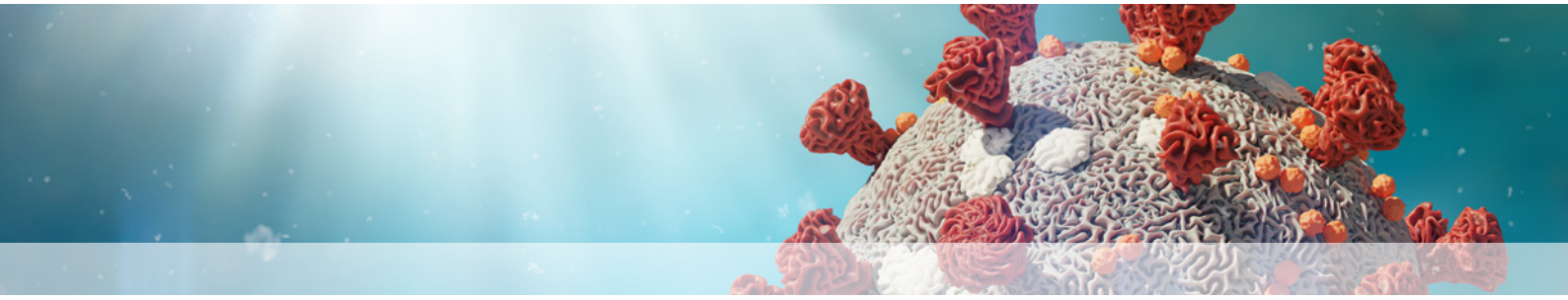
MACHEREY-NAGEL

NucleoMag® Virus

Viral nucleic acid isolation from saliva and respiratory swab samples – Enlarged number of extractions



Protocol details

Application	Viral DNA / RNA isolation from saliva and respiratory swabs
Kit	NucleoMag® Virus
REF	744800.4
Sample material and input	Saliva and respiratory swab samples – Sample input 200 µL
Kit size	Suitable for 8 x 96 preps
Protocol modifications	Optimized volumes to achieve (8 x 96 / 768) preps instead of (4 x 96 / 384) preps

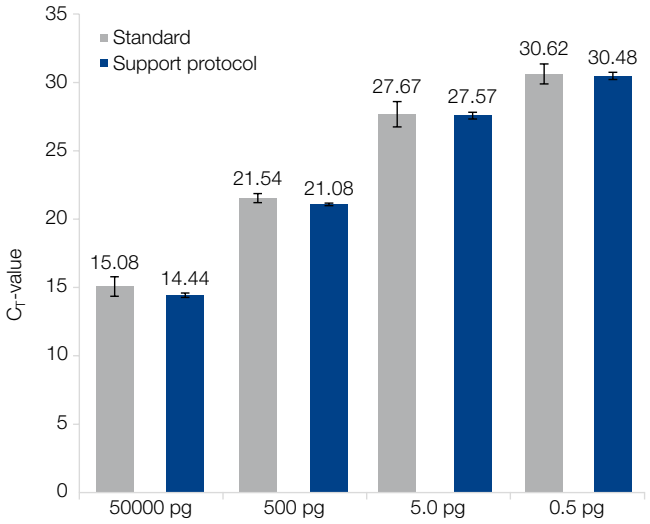
Specifications and protocol limitations

The here described support protocol has been developed to increase sample throughput for the extraction of viral nucleic acids of saliva and respiratory swab samples. Buffer volumes have been redesigned in order to achieve the maximum number of reactions per kit. The protocol is only intended for human saliva and respiratory swabs and has not been verified with other sample materials.

Protocol steps

Procedure	
1 Lyse samples	Provide 200 µL of each sample in a suitable reaction vessel. Add the following reagents to each sample and mix: 130 µL Lysis Buffer MVL 2 µL Carrier RNA 5 µL Proteinase K Incubate for 10 min at 56 °C with moderate shaking or mixing (e.g. 800 rpm).
2 Bind viral RNA / DNA to magnetic beads	Add 15 µL resuspended NucleoMag® V-Beads and 350 µL Binding Buffer MV2 and shake for 5 min at room temperature or mix well by repeated pipetting up and down. Separate the magnetic beads against the side of the tube or plate by using a suitable magnetic separator. Wait at least 2 min until all the beads have been attracted to the magnet. Remove and discard the supernatant by pipetting.
3 Wash with MV3	Add 350 µL Wash Buffer MV3 and resuspend the beads by shaking or pipetting up and down. Separate the magnetic beads. Remove and discard the supernatant by pipetting.
4 Wash with MV4	Add 350 µL Wash Buffer MV4 and resuspend the beads by shaking or pipetting up and down. Separate the magnetic beads. Remove and discard the supernatant by pipetting.
5 Air dry magnetic beads	Air dry the magnetic bead pellet for 10 min at room temperature.
6 Elution	Add 50–75 µL of Elution Buffer MV6 to each well or tube and resuspend the beads by shaking 5 min at 56 °C. Alternatively,resuspend beads completely by repeated pipetting up and down and incubate for 5 min at 56 °C. Separate the magnetic beads. Transfer the supernatant to a suitable vessel for further analysis.

Application data



Reliable and comparable detection of T7 bacteriophage DNA in human saliva compared to standard protocol

T7 bacteriophage DNA was spiked into human saliva (200 µL) in a 4-fold dilution series and isolated using the NucleoMag® Virus using standard protocol and support protocol for saliva and respiratory swab samples (see protocol steps). qPCR analysis was performed with a Taqman® probe for T7 DNA. T7 bacteriophage DNA was detected consistently and reliably over a range of a fourfold dilution series. Comparing the C_T-values from the support protocol for saliva and respiratory swab samples with the standard protocol, the results show comparable recovery.

Differences at a glance

The following table lists the differences in volumes between the standard protocol of the NucleoMag Virus Kit (REF 744800.4) and the support protocol for saliva and respiratory samples.

		Standard protocol	Saliva and respiratory sample protocol
1	Lysis Buffer MVL	200 µL	130 µL
2	Binding Buffer MV2	600 µL	350 µL
3	Wash Buffer MV3	500 µL	350 µL
4	Wash Buffer MV4	500 µL	350 µL
5	Wash Buffer MV5 (Rinse)	550 µL	obsolete due to drying step
6	Elution Buffer MV6	100 µL	75 µL
7	NucleoMag® V-Beads	30 µL	15 µL
8	Carrier RNA (reconstituted)	4 µL	2 µL
9	Proteinase K (reconstituted)	10 µL	5 µL
10	Carrier RNA Buffer	for reconstitution	unchanged
11	Proteinase Buffer PB	for reconstitution	unchanged

Remarks for automated use

Please contact our technical support Bioanalysis (support@mn-net.com) regarding automation inquiries. Main changes of the protocol include:

- Adjusted volumina as stated in the protocol steps
- Replacement of Rinsinge step with Wash Buffer MV5 by air drying the magnetic bead for 10 min (step 5)

Ordering information

Kit	REF	Pack of
NucleoMag® Virus	744800.4	384 preps using the standard protocol 768 preps using the support protocol

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