MACHEREY-NAGEL

NucleoMag® Virus

Viral nucleic acid isolation from saliva and respiratory swab samples NucleoMag® Virus – Fast Track

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	Streamlined protocol with less hands-on-time and optimized volumes to achieve increased number preparations: 8 x 96 / 768 preps instead of (4 x 96 / 384 preps) preps

Specifications and protocol limitations

The here described support protocol has been developed to increase sample throughput with less hands-on time by combining lysis and binding step 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

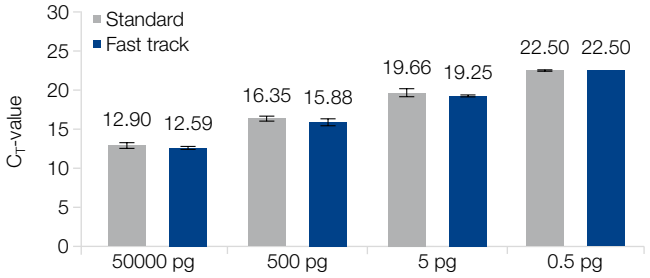
Procedure	
1 Lyse samples	Provide 200 µL of sample in a suitable reaction vessel. Add the following reagents to each sample and mix: 130 µL Lysis Buffer MVL 2 µL Carrier RNA stock solution 5 µL Proteinase K 350 µL Binding Buffer MV2 15 µL NucleoMag® V-Beads For higher convenience a premix of the above mentioned components can be prepared. This premix (502 µL per sample) should be added to the sample immediately (within 15 min after preparation). Please refer to the table below for instructions of premix preparation for different sample sizes. Incubate for 10 min at 56 °C with moderate shaking or mixing (e.g. 800 rpm). Continue to incubate for 5 min at RT with moderate shaking.
2 Separate magnetic beads	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 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.

Preparation of lysis / binding premix (optional)

Sample number	1 x	8 x	48 x	96 x
Lysis Buffer MVL	130 µL	1040 µL	6240 µL	12480 µL
Carrier RNA	2 µL	16 µL	96 µL	192 µL
Proteinase K	5 µL	40 µL	240 µL	480 µL
Binding Buffer MV2	350 µL	2800 µL	16800 µL	33600 µL
NucleoMag® V-Beads	15 µL	120 µL	720 µL	1440 µL
Total volume	502 µL	4016 µL	24096 µL	48192 µL

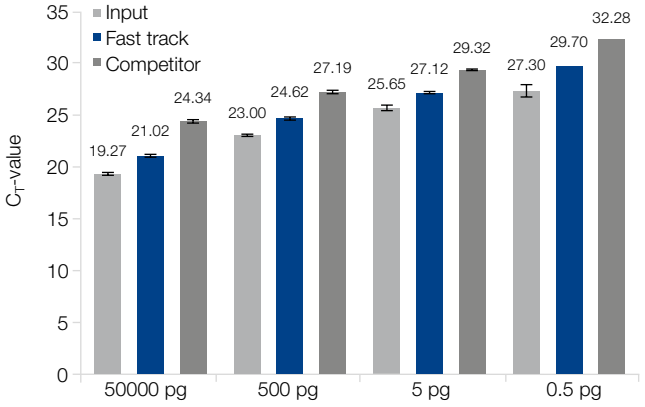
For higher convenience a premix of the above mentioned components can be prepared. This premix should be added to the sample immediately (within 15 min after preparation).
Lysis Buffer with added Carrier RNA can be stored at room temperature for 1–2 weeks.

Application data



Reliable and comparable detection of T7 bacteriophage DNA in human saliva using the fast track protocoll

T7 bacteriophage DNA was spiked into human saliva (200 µL) in a 4-fold dilution series and isolated using the NucleoMag® Virus standard protocol and fast track 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 fast track protocol for saliva and respiratory swab samples with the standard protocol, the results show comparable recovery.



Higher recovery of MS2 bacteriophage RNA in human saliva compared to competitor

Analogous to the detection of T7 DNA, MS2 bacteriophage RNA was spiked into human saliva (200 µL) in a 4-fold dilution series. RNA was isolated using the NucleoMag® Virus fast track protocol for saliva and respiratory swab samples (Fast Track) and Competitor Kit T. As a recovery control, the same amount of RNA was spiked into elution buffer and not purified (Input). qRT-PCR analysis was performed with a Taqman® probe for MS2 RNA. MS2 RNA was detected consistently and reliably over a range of a fourfold dilution series. Comparing the C_T-values from the fast track protocol for saliva and respiratory swab with the competitor kit T, a better recovery was obtained by our fast track protocol.

Differences at a glance

The following table lists the difference in the working steps between the standard protocol of the NucleoMag® Virus Kit (REF 744800.4) and the fast track protocol for saliva and respiratory samples

		Standard protocol	Fast track protocol for saliva and respiratory samples
1	Lysis	200 µL sample 200 µL MVL 4 µL Carrier RNA 10 µL Proteinase K	200 µL sample 130 µL MVL 2 µL Carrier RNA 5 µL Proteinase K 350 µL MV2 15 µL V-Beads
2	Lysis incubation	10 min at 56 °C	10 min at 56 °C
3	Binding	600 µL MV2 30 µL V-Beads	Included in step 1
4	1st wash	500 µL MV3	350 µL MV3
5	2nd wash	500 µL MV4	350 µL MV4
6	Rinse / dry	550 µL MV5	10 min at RT
7	Elution	50–100 µL MV6	75 µL MV6

Remarks for automated use

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

- Binding Buffer MV2 and NucleoMag® V-Beads are directly supplied to lysis reagents to reduce hands-on-time
- Adjusted volumina as stated in the protocol steps
- Replacement of Rinsing 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	768 preps using the fast track protocol

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