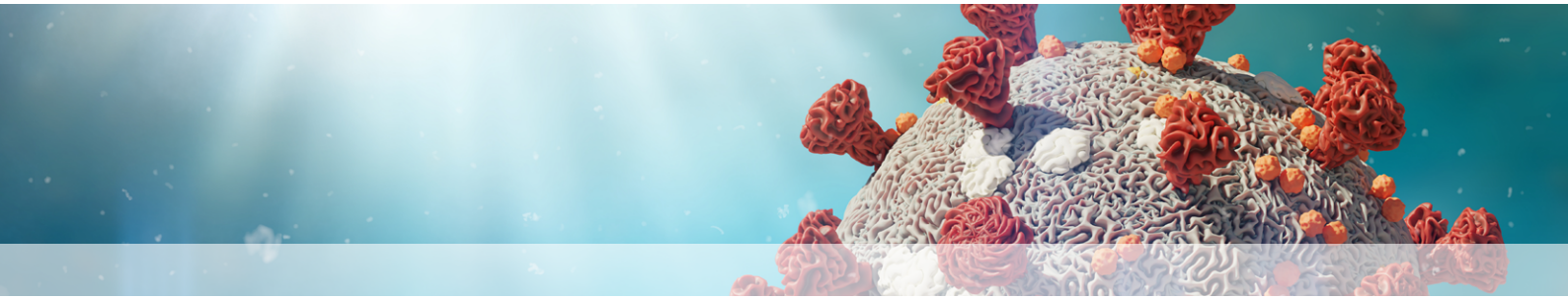
MACHEREY-NAGEL

NucleoSpin® 96 Virus

96-well

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



Protocol Details

Application	Viral DNA/RNA isolation from saliva and respiratory swabs
Kit	NucleoSpin® 96 Virus & NucleoSpin® 96 Virus Core kit
REF	740691.2 / 740691.4 / 740452.4
Sample Material and Input	Saliva and respiratory swab samples – Sample Input 200 µL
Kit Size	2x96 preps, 4x96 preps, 4x96 preps (Core Kit)
Information	This support protocol describes a fast protocol of the NucleoSpin® 96 Virus kit using up to 200 µL respiratory swab wash solution or saliva.

Specifications and protocol limitations

The here described support protocol has been developed to increase sample throughput for the extraction of viral nucleic acids from saliva and respiratory swab samples. Buffer volumes and sample amount have been adjusted to allow a single step for sample transfer and binding while at the same time enableling the input of up to 200µL respiratory swab wash solution or saliva. Furthermore, the protocol was streamlined to allow a faster processing time and easier handling during the wash steps. The protocol is only intended for human saliva and respiratory swabs and has not been verified with other sample materials. Also check our protocol recommendations for respiratory samples under the COVID-19 section of our homepage when preparing swabs.

Protocol Steps

Procedure	
1 Before starting	Check if Buffer RAV1, Buffer RAV3 and Proteinase K were prepared according to section 3 of the respective user manual.
2 Lyse samples	Provide 200 µL of each sample in a suitable reaction vessel. Add 400 µL Lysis Buffer RAV1 and 20µL Proteinase K to each sample and mix. Incubate for 10 min at room temperature with moderate shaking.
3 Adjust viral nucleic acid binding conditions	Add 400µL ethanol (96–100 %) to each lysate and mix them by pipetting up and down 4–5 times.
4 Transfer samples to binding plate	Setup the vacuum manifold for Binding / Washing steps according to the NucleoSpin® 96 Virus user manual. Transfer each of the samples to the wells of a NucleoSpin® 96 Virus Binding Plate. Do not moisten the rims of the individual wells while dispensing the samples. Seal the unused wells of the NucleoSpin® 96 Virus Binding Plate with Self-adhering PE Foil.
5 Bind viral nucleic acids to silica membrane	Apply vacuum of -0.2 to -0.4 bar* to allow the samples to pass through the membrane (2–5min). Flowthrough rate should be about 1–2 drops per second. Adjust vacuum strength accordingly.
6 1st Wash Wash Buffer RAW	Add 500 µL Wash Buffer RAW to each well of the NucleoSpin® 96 Virus Binding Plate. Apply vacuum (-0.2 to -0.4 bar*) until all buffer has passed through the well of the NucleoSpin® 96 Virus Binding Plate (2–5min). Release vacuum.
7 2nd Wash Wash Buffer RAV3	Add 700 µL Wash Buffer RAV3 to each well of the NucleoSpin® 96 Virus Binding Plate. Apply vacuum (-0.2 to -0.4 bar*) until all buffer has passed through the well of the NucleoSpin® 96 Virus Binding Plate (2–5min). Release vacuum.

Procedure		
8	Remove MN Wash Plate	After the final washing step close the valve, relase the vacuum and remove the MN Wash Plate as well as the waste container from the inside of the vacuum manifold. Place a clean waste container inside the vacuum manifold then place back the manifold lid and put NucleoSpin® 96 Virus Binding Plate back on top.
9	Dry silica membrane	Dry the membrane by applying -0.6 bar* for 15 minutes.
10	Elution	Dispense 100–120 µL RNase-free water or Elution Buffer RE directly onto the membrane of each well. Incubate at room temperature for 2–3 minutes. Apply vacuum of -0.4 bar* until all of the samples have passed. Optional: Repeat elution step once

* Reduction of atmospheric pressure

Storage of carrier RNA in Buffer RAV1

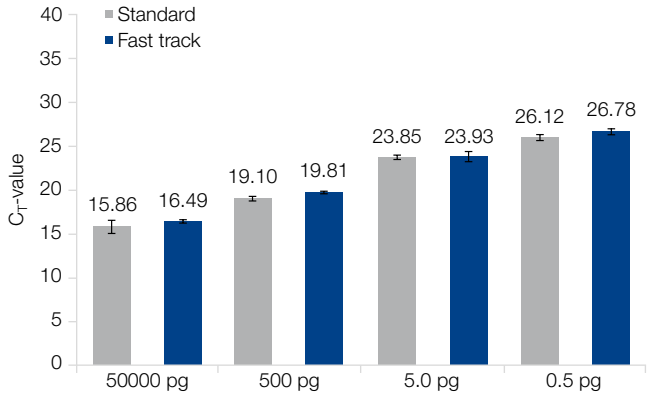
Storage conditions

Buffer RAV1 with Carrier RNA can be stored at room temperature for 1–2 weeks. Storage at room temperature prevents salt precipitation and avoids preheating of the buffer solution!

For storage for up to 4 weeks storage of Buffer RAV1 with added Carrier RNA at 4 °C is recommended.

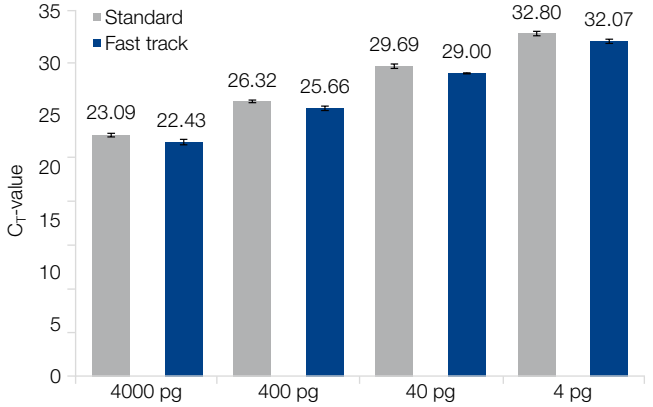
For long time storage Buffer RAV1 with added Carrier RNA can be stored in aliquots at -20 °C. Storage at 4 °C or below may cause salt precipitation. Therefore, the mixture must be preheated at 40–60 °C for a maximum of 5 min in order to dissolve precipitated salts.

Application Data



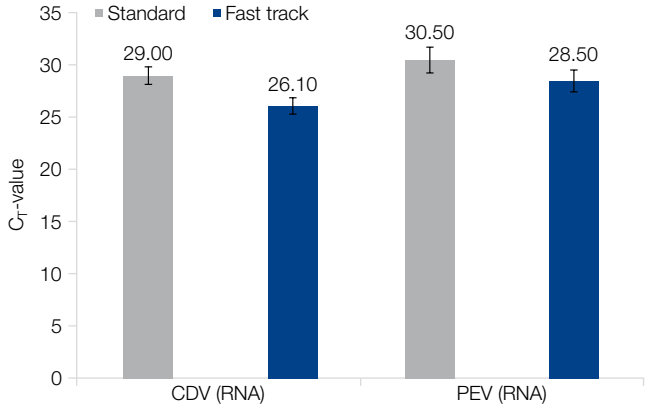
Reliable and comparable detection of T7 bacteriophage DNA in human saliva using the fast track protocoll

T7 bacteriophage DNA was spiked into human saliva (200 µL) in a 4-fold dilution series and isolated using the NucleoSpin® 96 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 Ct-values from the fast track protocol for saliva and respiratory swab with the standard protocol, the results show comparable recovery.



Reliable and comparable detection of MS2 bacteriophage RNA in human saliva using the fast track protocoll

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 NucleoSpin® 96 Virus standard protocol and fast track protocol for saliva and respiratory swab samples (see protocol steps). 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 Ct-values from the fast track protocol for saliva and respiratory swab with the standard protocol, the results show comparable recovery.



Detection of Canine distemper virus (CDV) and Porcines Enterovirus (PEV) in cattle saliva

In further experiments, the standard protocol and the fast track protocol for saliva and respiratory swabs were used for the isolation and detection of CDV and PEV nucleic acids (both RNA viruses) in bovine saliva by qRT-PCR analysis.

For both virus detections, lower Ct-values were obtained using fast track protocol compared to the standard protocol. Thus, the fast track protocol is suitable for both human and animal bovine saliva.

Protocol differences at a glance

The following table lists the differences between the standard protocol of the NucleoSpin® 96 Virus Kit (REF 740691.2 / 740691.4 / 740452.4) and the fast track protocol for saliva and respiratory samples (without vacuum steps).

		Standard protocol	Fast track protocol for saliva and respiratory sample
1	Lysis	100 µL sample	200 µL sample
		400 µL RAV1	400 µL RAV1
		20 µL Proteinase K	20 µL Proteinase K
		10 min RT	10 min RT
2	Adjust binding conditions	400 µL EtOH	400 µL EtOH
3	Loading	Transfer up to 1 mL	Transfer up to 1 mL
4	1st Wash	500 µL RAW	500 µL RAW
5	2nd Wash	700 µL RAV3	700 µL RAV3
6	3rd Wash	700 µL RAV3	obsolete
7	Drying	15 min	15 min
8	Elution	100 µL RE	100 µL RE

Remarks for automated use

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

- Adjusted sample volumina
- Exclusion of 3rd wash step (RAV3)

Ordering information

Ordering information		
Kit	REF	Pack of
NucleoSpin® 96 Virus	740691.2	192 preps
	740691.4	384 preps
	740452.4	384 preps (Core Kit)

Disclaimer