Isolation kits, viral nucleic acid, viral RNA, NucleoSpin® RNA Virus

LE

MACHEREY-NAGEL

For purification of viral RNA and DNA from serum, plasma and cell-free biological fluids.

- Silica membrane technology
- Typical recovery: >90%
- Sample material: 150µL serum, plasma, cell-free biological fluids
- Fragment size: 100bp to 30kb
- Elution volume: 50µL
- Binding capacity: 40µg
- Preparation time: 30min/4-6 preps
- Format: mini spin column
- Support protocol for parallel isolation of viral RNA and DNA (proteinase K required)
- Applicable for HCV, HBV, HAV, HIV, HSV, HPV, VZV, EBV, parvovirus B19, H5N1

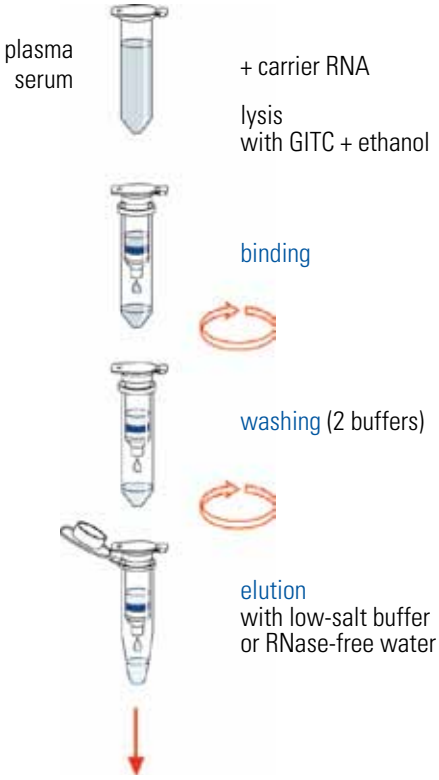
NucleoSpin® RNA Virus is designed for the isolation of viral nucleic acids from serum, plasma or any cell-free biological fluids, featuring a special type of membrane which has a high binding capacity for nucleic acids.
The procedure is rapid and easy. The lysate is incubated for 5 to 10min and applied to the NucleoSpin® column. After two washing steps to remove contaminations the RNA can be eluted with water or low-salt buffer. The kit contains all necessary buffers, spin columns and detailed protocols. Generally, NucleoSpin® RNA Virus is suited for the parallel purification of viral RNA and DNA (support protocol). For example, isolation of HBV DNA is performed with an additional proteinase K digest (enzyme not included in the kit). For isolation of viral DNA with highest sensitivities, the NucleoSpin® Blood kit is recommended.

Kit components: NucleoSpin® RNA Virus columns, 2mL collecting tubes, buffers, RNase-free water, carrier RNA.

Catalogue No	Alt. No	Preps per kit	Pack qty
NZ74095610	740956.10	10	1
NZ74095650	740956.50	50	1
NZ740956250	740956.250	250	1

Catalogue No	Alt. No	Description
NZ740506	740506	Proteinase K, 100mg

NucleoSpin® RNA Virus procedure



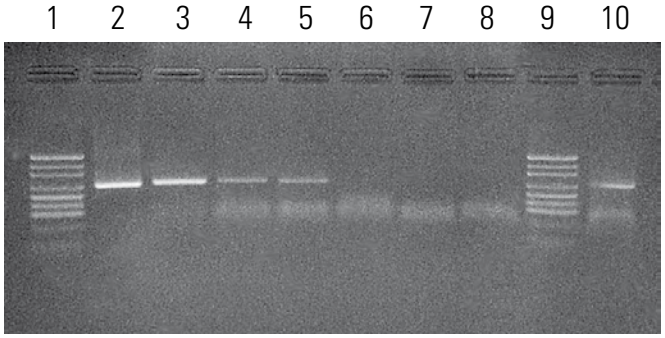
Purification of HCV RNA with NucleoSpin® RNA Virus – analysis with primary and subsequent nested PCR*
A HCV positive clinical specimen was quantitatively determined by Cobas Amplicor HCV assay.
According to the results several dilutions were produced with negative normal serum for testing NucleoSpin® RNA virus sensitivity. In detail, starting with about 50,000 copies HCV/mL serum samples were diluted down to theoretically about 30 copies/mL. Afterwards, diluted samples were purified according to the NucleoSpin® RNA Virus procedure. From 50µL eluate 5µL were used for PCR* (figure A) and subsequent nested PCR* (figure B) amplification procedure. PCR* products were analysed by agarose gel electrophoresis.
With standard PCR* about 1000 copies per mL can be detected. After nested PCR* even highly diluted samples containing theoretically about 30 to 60 copies per mL gave positive signals. Positive and negative controls were analysed in parallel for verification of the test system. Results indicate that NucleoSpin® RNA Virus procedure shows high recovery rates even for low–titre samples and the purified RNA is suitable for appropriate investigations (e.g., sample pooling).
Data kindly provided by DR C Tiemann, Laboratory Prof. Hagedorn, Herford, Germany

A: Primary PCR*
1: Marker (pUC19-MspI)
2: HCV – 50,000cp/mL
3: HCV – 5,000cp/mL
4: HCV – 2,500cp/mL
5: HCV – 1,250cp/mL
6: HCV – 500cp/mL
7: HCV – 250cp/mL
8: Negative control
9: Marker (pUC19 – MspI)
10: Positive control

B: Nested PCR*
1: Marker (pUC19 – MspI)
2: HCV – 5,000cp/mL
3: HCV – 500cp/mL
4: HCV – 250cp/mL
5: HCV – 125cp/mL
6: HCV – 62.5cp/mL
7: HCV – 31.25cp/mL
8: Negative control
9: Marker (pUC19-MspI)
10: Positive control

*Polymerase Chain Reaction (PCR) is a process covered by patents owned by Hoffman-La Roche

A: primary PCR*



B: nested PCR*