MACHEREY-NAGEL

# NucleoSpin® 96 Virus

## Automated viral DNA and RNA purification on the Biomek 4000 workstation

## Introduction

The reliable and fast identification of viral strains from clinical samples plays a vital role in infectious disease research and related applications. PCR assays have become the method of choice as they deliver fast and reliable results. However, these assays require viral DNA or RNA isolated from a given biological sample. Therefore, MACHEREY NAGEL offers a range of products for purification of viral nucleic acids.

To manage processing of increasing sample numbers and enable automated workflows MACHEREY-NAGEL has developed the NucleoSpin® 96 Virus kit. The kit allows isolation of viral DNA and / or RNA from up to 96 samples in parallel. The NucleoSpin® 96 Virus kit can be automated on liquid handling platforms equipped with a vacuum manifold for 96 well purification plates.

In this application note we demonstrate the automated purification of nucleic acids from samples spiked with MS2 bacteriophage RNA using the NucleoSpin® 96 Virus kit on a Biomek 4000 workstation. The protocol enables the reliable and sensitive detection of sample RNA in downstream qPCR assays. In addition, we show the absence of cross contaminations using HeLa cells as an exemplary sample material.

Example configuration of the Biomek 4000 workstation

## Product at a glance

| NucleoSpin® 96 Virus | |
|---|---|
| Technology | Silica membrane technology |
| Sample material | ≤ 150 µL cell-free biological fluid (e.g.,plasma) |
| Fragment size | 100 bp–approx. 50 kbp |
| Typical recovery | > 90 % |
| Elution volume | 70–100 µL |

| Biomek 4000 | |
|---|---|
| Technology | Automated liquid handling platform |
| Capacity | Low to medium throughput; 12 deck positions |
| Pipetting volume | 1–1000 µL |
| Equipment and features | 1/8-channel pipetting tool, gripper, vacuum manifold |

## Material and methods

Three experiments were performed to assess important questions related to viral nucleic acid isolations from clinical samples:

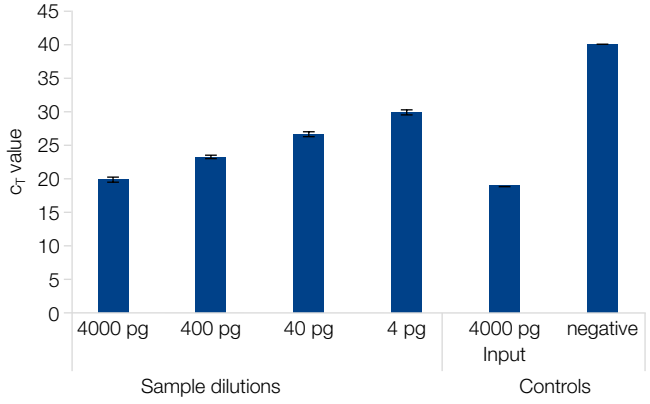
1. Sensitivity
2. Reproducibility
3. Absence of cross-contaminations

In order to measure sensitivity of the qPCR assay, samples were spiked with recombinant MS2 phage RNA ranging from 4 pg to 4000 pg. Samples were then subjected to the NucleoSpin® 96 Virus standard protocol using a customized script on the automated Biomek 4000 workstation. A qRT-PCR analysis was performed with custom primers for a MS2-specific target transcript.

Reproducibility was measured by performing isolations from samples spiked with carrier RNA and determining RNA yield photometrically.

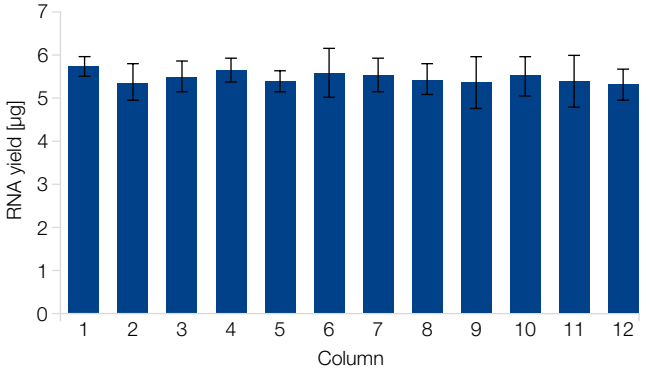
The absence of cross-contaminations was confirmed by performing qPCR (ß-actin gene) with isolates obtained from HeLa cells (1 x $10^5$ cells; positive control) and PBS (negative control) arranged in a checkerboard pattern.

## Application data



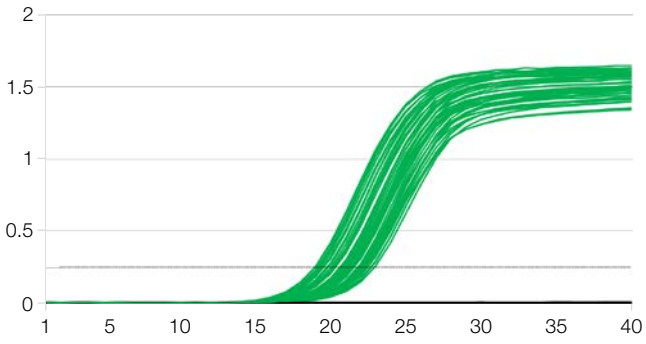

qRT-PCR analysis of MS2 RNA recovered from human plasma

Recombinant MS2 RNA was spiked into samples in a serial dilution ranging from 4000 pg down to 4 pg. MS2 RNA was recovered from the samples as well as a positive control (4000 pg input) and a negative control using the NucleoSpin® 96 Virus kit on the Biomek 4000 automated workstation. Sensitivity was determined by a subsequent qRT-PCR assay using custom primers for a MS2-specific transcript.




RNA recovery from samples spiked with Carrier RNA

Samples were spiked with recombinant Carrier RNA. RNA was recovered using the NucleoSpin® 96 Virus kit on the Biomek 4000 automated workstation. RNA concentrations were determined by UV spectrophotometry in order to demonstrate the high reproducibility of the RNA purification process.




Cross-contamination analysis using qPCR

Nucleic acids were isolated from HeLa cells (1 x $10^5$ cells/sample) and negative control samples (PBS) arranged in a checkerboard pattern using the NucleoSpin® 96 Virus kit on the Biomek 4000 automated workstation. Absence of signal in the negative control samples indicates a cross-contamination free nucleic acid isolation procedure.

## Automate your viral nucleic acid extraction from cell-free biological fluids

MACHEREY-NAGEL developed a script for the Biomek 4000 automated liquid handling workstation enabling the use of NucleoSpin® 96 kits on this system.

Here we show the compatibility of the Biomek 4000 platform with the NucleoSpin® 96 Virus kit and demonstrate the suitability of this workflow for sensitive, cross-contamination free molecular testing assays:

- Excellent sensitivity of downstream qPCR assays using DNA / RNA isolated with the NucleoSpin® 96 Virus kit
- Reliable performance and reproducible recovery of nucleic acids, e.g. viral RNA from human plasma
- Cross-contamination free, automated purification procedure

## References

(1) Abdelnabi et al., 2019 "A novel druggable interprotomer pocket in the capsid of rhino- and enteroviruses.", PLoS Biology

(2) Dickson et al., 2017 "Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector.", Science Advances

(3) Marcombe et al., 2017 "Insecticide resistance status of malaria vectors in Lao PDR.", PLoS One

## Ordering information

| Product | Specifications | Pack of | REF |
|---|---|---|---|
| NucleoSpin® 96 Virus | 96-well silica membrane kit for the isolation of viral DNA / RNA from cell-free biological fluids | 2 x 96 preps<br>4 x 96 preps | 740691.2<br>740691.4 |
| NucleoVac 96 | Vacuum manifold, spacer set | 1 | 740681 |
| NucleoSpin® 96 Virus Core Kit | 96-well silica membrane kit with basic content for the isolation of viral DNA / RNA from cell-free biological fluids | 4 x 96 preps | 740452.4 |
| NucleoSpin® 8 Virus | 8-well strip silica membrane kit for the isolation of viral DNA / RNA from cell-free biological fluids | 12 x 8 preps<br>60 x 8 preps | 740643<br>740643.5 |