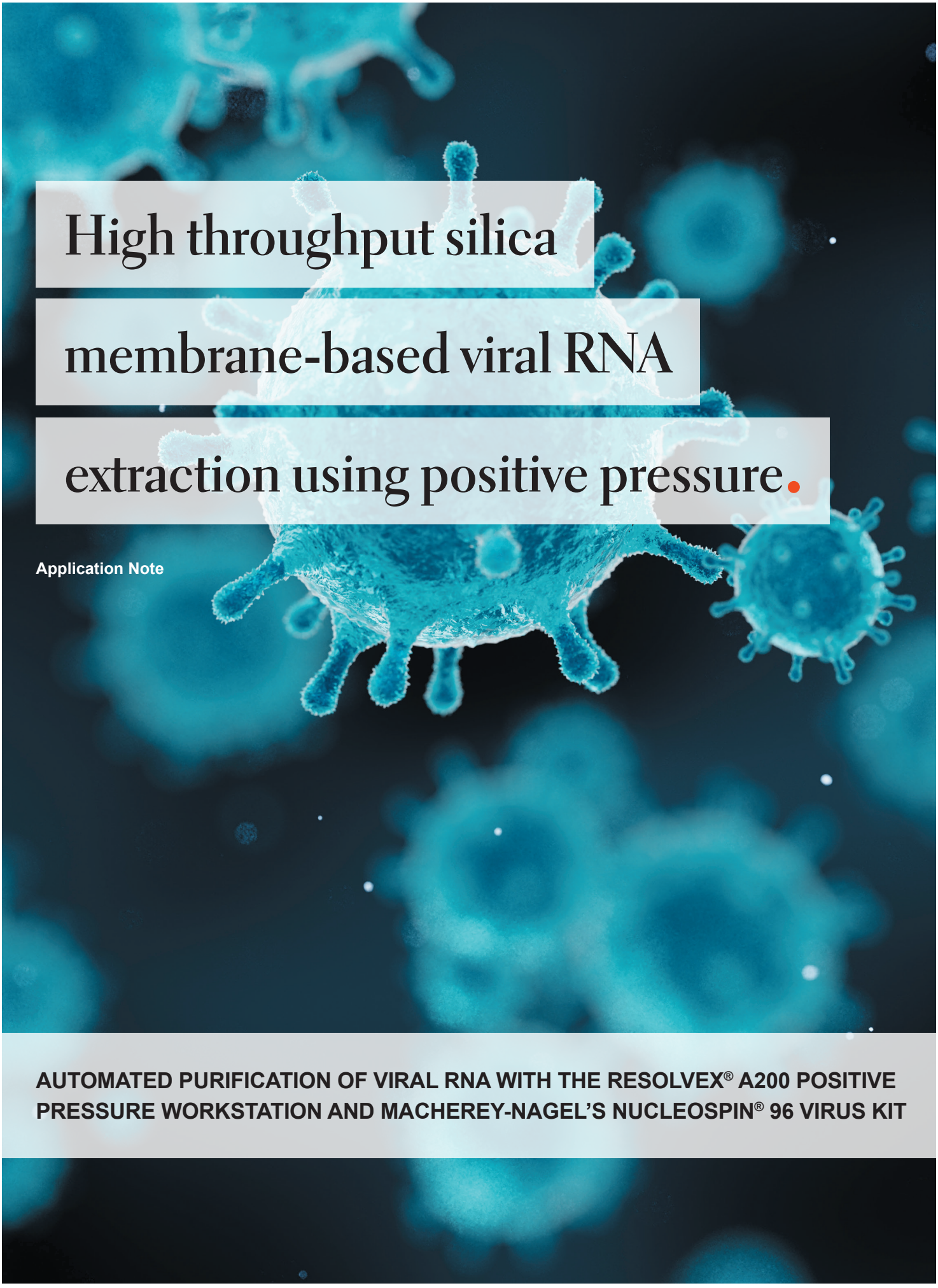

High throughput silica membrane-based viral RNA extraction using positive pressure.

Application Note

AUTOMATED PURIFICATION OF VIRAL RNA WITH THE RESOLVEX® A200 POSITIVE PRESSURE WORKSTATION AND MACHEREY-NAGEL'S NUCLEOSPIN® 96 VIRUS KIT

INTRODUCTION

The global COVID-19 pandemic has resulted in a rapid increase in demand for the isolation of viral RNA. The required throughput for the extraction of viral RNA from swab samples quickly rose to levels that could not be handled using predominantly manual or low throughput automated workflows.

The NucleoSpin 96 Virus kit from MACHEREY-NAGEL is designed for the extraction of viral nucleic acids – RNA and DNA viruses – from cell-free, biological fluids, such as saliva, urine, serum or plasma, and sample homogenates (particle-free supernatants), for example, from swabs, tissue or stool. It uses a purification process based on binding of nucleic acids to a silica membrane column in a high throughput 96-well format. The NucleoSpin 96 silica membrane extraction plates can be processed using vacuum or positive pressure in an automated laboratory set-up.

Combining the NucleoSpin 96 plate-based workflow with the Resolvex A200 workstation enables processing of silica membrane extraction plates under positive pressure, offering an accessible semi-automated solution to rapidly scale up throughput and reduce hands-on time.

MATERIALS AND METHODS

This application note describes the semi-automated extraction workflow for spiked viral RNA and DNA samples using the NucleoSpin 96 Virus kit on a Resolvex A200 positive pressure workstation. The Resolvex A200 can also be integrated into a Freedom EVO® workstation to extend walkaway time.

Resolvex A200 instrument configuration

NucleoSpin 96-well silica membrane extraction plates were processed on the Resolvex A200 96 positive pressure workstation (Figure 1), using a dedicated set of adapters comprising a Slotted Spacer BGO Gold, a Red Rise High Spacer BGO and Barrier Long Profile Inserts (Figure 2) to allow binding, washing and elution to be performed on the system.

The required buffers were prepared in bottles and connected to the dispenser. Optimal working input pressure – air or nitrogen – is 5.5 bar (80 psi).

Figure 1: Resolvex A200 positive pressure workstation.

Up to 100 µl liquid sample material is mixed with Proteinase K (optional) and Lysis Buffer RAV1 (please refer to the NucleoSpin 96 Virus kit manual for detailed information) for binding of nucleic acids to the silica membrane column. Contaminants and salts are subsequently removed by washing with Wash Buffer RAW (once) and Wash Buffer RAV3 (twice). After drying, highly pure nucleic acids are eluted under low ionic strength conditions using the slightly alkaline Elution Buffer RE.

NucleoSpin 96 Virus kit	
Technology	96-well silica membrane extraction plate
Sample material	Cell-free, biological fluids (e.g. saliva, plasma, serum, urine), swab wash solutions, sample homogenates (particle-free supernatants, eg. from stool and tissue)
Sample volume	100 µl
Target molecules	Viral RNA and DNA
Fragment size	100 bp to approx. 50 kbp
Elution volume	70-100 µl

Table 1: Kit specifications.

Workflow overview:

Workflow	
Step 1	96-well silica membrane extraction plate
	Add lysis buffer RAV1 to samples for lysis in a biosafety cabinet and incubate for 10 minutes.
Step 2	Add 400 µl 99 % ethanol to adjust binding conditions.
Step 3	Transfer lysed samples to the NucleoSpin 96 silica membrane extraction plate.
Step 4 Resolvex A200	Assemble processing stack according to Figure 2, with the silica membrane extraction plate on top of the red spacer containing a Barrier Long Profile Insert (splash guard insert for the Red Rise High Spacer), and place in the Resolvex A200.
Step 5 Resolvex A200	Dispense binding buffer RAV1.
Step 6 Resolvex A200	Load sample onto the silica membrane extraction plate using a pressure gradient.
Steps 7-9 Resolvex A200	Wash the silica membrane using buffers RAW (1x) and RAV3 (2x) and optimized pressure profiles.
Step 10	Park the NucleoSpin 96 silica membrane extraction plate on a sheet of filter paper. Replace Barrier Long Profile Insert*. Reassemble the processing stack.
Step 11 Resolvex A200	Dry the silica membrane under constant pressure.
Step 12 Resolvex A200	Reassemble the plate stack according to Figure 3. Place the elution plate (eg. a deep-well plate) under the stack.
Step 13 Resolvex A200	Dispense elution buffers using pressure gradients. Collect eluate in elution plate.

Table 2: Schematic overview of the NucleoSpin 96 Virus workflow on the Resolvex A200.
*Gently tapping the plate on the sheet of filter paper is recommended to remove residual droplets. Barrier Long Profile Insert (splash guard) may be reused depending on your workflow.

After lysis, the sample is transferred onto the NucleoSpin 96 silica membrane extraction plate, and the processing stack is assembled as shown in Figure 2, with the plate on top of a Spacer Slotted BGO Gold and Red Rise High Spacer to maintain the correct distance between the plate and waste, and placed into the Resolvex A200. The Red Rise High Spacer contains a Barrier Long Profile Insert (splash guard) to prevent cross-contamination from one silica membrane extraction plate nozzle to the next. Binding and washing steps are performed using this stack assembly, employing pressure gradients to ensure even processing across the plate. For partial plate runs, it is recommended that the unused part of the plate is sealed.

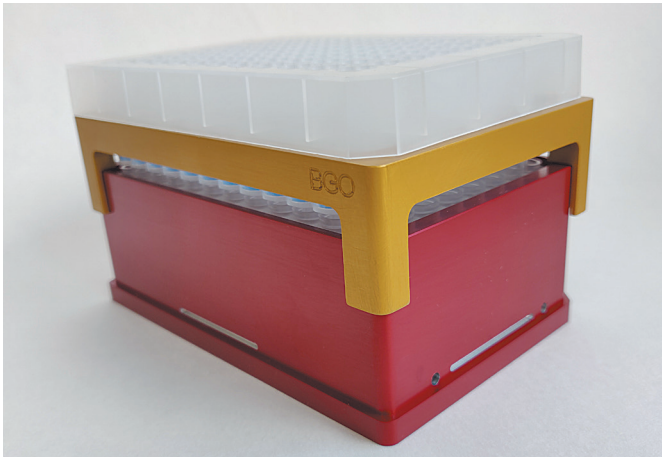

Figure 2: Processing stack assembly used for binding and washing steps comprising a Slotted Spacer BGO Gold, a Rise High Spacer BGO Red and a Barrier Long Profile Insert.

Prior to elution, the stack is rearranged as depicted in Figure 3.

Figure 3: Elution stack assembly used for the elution step.

RESULTS AND DATA ANALYSIS

qRT-PCR analysis of MS2-phage RNA recovered from PBS/saline

MS2-phage RNA was spiked into 100 µl sample solution in a dilution series ranging from 4,000 pg down to 4 pg (1:10 dilutions, n = 3 for each dilution) and recovered using the NucleoSpin 96 Virus kit on the Resolvex A200 workstation. Sensitivity was determined by a subsequent qRT-PCR assay with a TaqMan® PCR probe for MS2-phage RNA (Roche) using the SensiFAST™ Probe Lo-ROX One-Step Kit (Bioline) on an Applied Biosystems® 7500 Real-Time PCR System. The method shown provides sensitive detection of phage RNA comparable to results obtained with alternative purification workflows, such as magnetic beads. The linearity of the average detection threshold over four log10 dilutions demonstrates the reliability of the RNA isolation process for different viral titers (Figure 4).

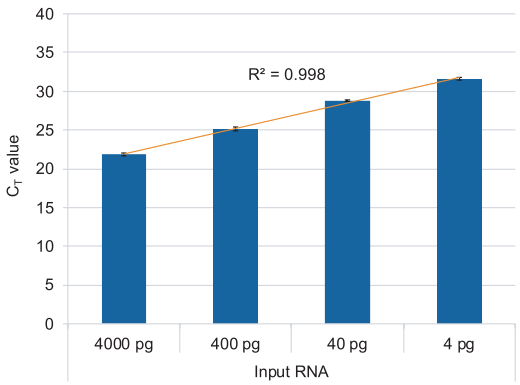

Figure 4: High sensitivity detection of MS2-phage RNA recovered from 100 µl liquid samples, 1:10 dilutions, n = 3 per dilution.

qRT-PCR analysis of Armored RNA® recovered from saliva/PBS samples

Amored RNA Enterovirus (Asuragen) (5 µl) was spiked into 95 µl saliva/PBS (1:1) samples in a serial dilution (1:4 dilutions, n = 3 per dilution). RNA was recovered using the NucleoSpin 96 Virus kit on the Resolvex A200 workstation. Sensitivity was determined by a subsequent qRT-PCR assay using the SYBR® Green Quantitative RT-qPCR Kit (Sigma-Aldrich) with primers for an armored RNA-specific transcript on a Bio-Rad CFX96 qPCR-Cycler. The target RNA could be detected over four orders of magnitude with excellent linearity (R^2 = 0.9911, Figure 5).

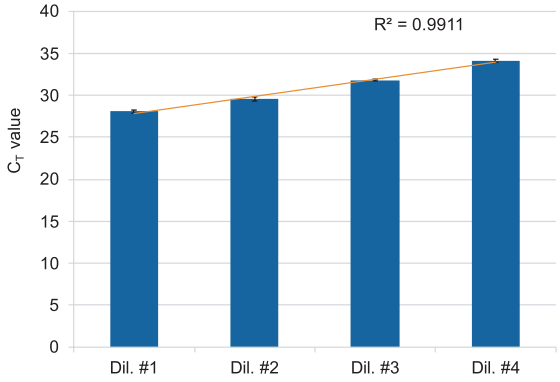

Figure 5: qRT-PCR analysis of Armored RNA Enterovirus recovered from spiked saliva/PBS samples (1:4 dilutions, n = 3 per dilution).

Absence of cross-contamination

Positive (T7 bacteriophage DNA) and negative (no DNA) control samples (100 µl of each) were arranged in a checkerboard pattern and subjected to the NucleoSpin 96 Virus kit procedure on the Resolvex A200 workstation. The eluates were examined for the presence of DNA by qPCR with a TaqMan PCR probe for T7 DNA (Roche) using the SensiFAST Probe Lo-ROX One-Step Kit (Bioline) on an Applied Biosystems 7500 Real-Time PCR System. Absence of a qPCR signal (Ct undetermined) in the negative control samples indicates a workflow free from cross-contamination (Figure 6).

SUMMARY

This joint solution from MACHEREY-NAGEL and Tecan delivers a robust protocol for high throughput extraction of viral RNA. Implementation of a Resolvex A200 positive pressure workstation reduces hands-on time significantly, allowing processing of 96 samples in approximately 60 minutes.

LEARN MORE

To learn more about Tecan's nucleic acid purification solutions, visit **www.tecan.com/NAP**
To learn more about Tecan's Resolvex A200, visit **www.tecan.com/ResolvexA200**
For more information on Tecan's solutions for COVID-19-related testing, please contact your sales representative or visit **www.tecan.com/COVID19**

ACKNOWLEDGEMENTS

This protocol was developed by MACHEREY-NAGEL application scientists and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application. Further information can be found at **https://www.mn-net.com/tecan**

Tecan Resolvex T7 phage DNA in PBS (positve control)

Cт	1	2	3	4	5	6	7	8	9	10	11	12
A	15,35	*	15,33	*	15,50	*	15,16	*	15,39	*	15,21	*
B	*	15,12	*	15,28	*	14,96	*	15,03	*	15,21	*	15,29
C	15,09	*	15,12	*	15,04	*	15,34	*	15,15	*	15,10	*
D	*	15,15	*	15,51	*	15,44	*	15,53	*	15,29	*	15,43
E	15,22	*	15,30	*	15,47	*	15,30	*	15,26	*	15,11	*
F	*	14,95	*	15,46	*	15,50	*	15,06	*	15,21	*	16,41
G	15,24	*	14,97	*	15,36	*	14,79	*	15,23	*	15,04	*
H	*	14,94	*	15,33	*	15,17	*	15,31	*	15,09	*	16,21
sample (Cт, positive controle)												
no sample (* Cт undetermined; negative controle)												

Figure 6: No amplification in the negative control wells indicates the absence of cross-contamination.

REFERENCES

[1] Tsalik et al (2018). New Molecular Diagnostic Approaches to Bacterial Infections and Antibacterial Resistance. Annual Review of Medicine 69, 379-394.

About the authors

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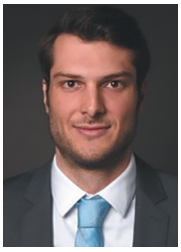

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