eppendorf

SHORT PROTOCOL No. 46

Automated isolation of viral nucleic acids using the NucleoMag® Virus kit on ep*Motion*® 5073m

Introduction

This protocol describes the automated process on the liquid handling system ep*Motion* 5073m using the NucleoMag Virus Kit from MACHEREY-NAGEL.

We show the configuration and pre-programmed method for automated purification of nucleic acids from cell-free body fluids, such as plasma or serum, for up to 24 samples.

Configuration and method procedure

Method name: NucleoMag_Virus_5073m.export
This protocol is programmed to process up to 24 samples in parallel on the ep*Motion* 5073m. The NucleoMag Virus kit is based on reversible adsorption of nucleic acids to magnetic beads under appropriate buffer conditions. The ep*Motion* 5073m is by default equipped with a Eppendorf ThermoMixer® (TMX) in combination with a magnetic separator allowing the entire process being performed without the need for labware transports. This protocol can be transferred to the bigger liquid handling system ep*Motion* 5075m as well.
Plasma samples spiked with viral RNA (MS2 phage RNA) or viral DNA (T7 phage DNA) (T7- and MS2-phages) are applied in volumes of 200 µL into 2.0 ml tubes in the PrepRack placed on the TMX (Fig. 1). To maximize the efficiency of nucleic acid recovery, it is recommended to use Eppendorf DNA LoBind tubes. The required volumes of buffers (Binding and Wash Buffers, Lysis and Elution Buffers, 80% ethanol) should be transferred respectively to 30 mL epMotion reservoirs. Proteinase K and resuspended NucleoMag V-Beads are positioned together in the reservoir rack tubes module within a reservoir rack on position B1 as described in Fig. 2. This automated protocol follows the procedure, which is recommended by MACHEREY-NAGEL. The protocol starts by adding Proteinase K and Lysis Buffer MVL to the samples, followed by an incubation step 10 min at 56°C and simultaneous mixing at 1200 rpm.
Afterwards, 600 µL of Binding Buffer MV2 and 30 µL NucleoMag V-Beads are added to the samples, followed by a 5 minutes mixing step.
A subsequent 2 min magnetic separation allows the complete accumulation of the NucleoMag V-Beads. After the magnetic bead separation, the supernatant is removed and discarded into the liquid waste tub. The DNA and RNA attached to the magnetic beads are washed twice: first with 500 µL of Wash Buffer MV3, followed by a removal of the supernatant. Then 400 µL of Wash Buffer MV4 are added, again followed by a removal of supernatant. A third washing step is subsequently performed with 550 µL of 80% ethanol and the supernatant is removed afterwards. The NucleoMag V-Beads are air dried to remove traces of ethanol for 7 min at 55°C while mixing at 1200 rpm. The genomic DNA is eluted from the NucleoMag V-Beads by adding 105 µL of Elution Buffer MV6, followed by mixing at 1300 rpm and 56°C for 5 min. It is possible to adjust the volume of the Elution Buffer MV6 according to the initial sample amount, to circumvent a strong dilution or concentration of eluted nucleic acids. After a final magnetic separation step for 2 min, 100 µL of the supernatant containing the purified viral DNA or RNA is transferred into fresh tubes in the Rack 24 on the C2 position.

Worktable Layout

Position	Item
TMX	PrepRack with DNA LoBind tubes 2.0 mL containing 200 µL blood sample. Be aware of the direction of tube placement, starting from position 1, 2, 3….
A2	50 µL filter tips
B1	Reservoir rack with 30 mL reservoirs and DNA LoBind tubes 2.0 mL containing reagents (Fig. 2)
B2	1000 µL filter tips
C2	Rack 24 with fresh LoBind tubes 2.0 mL for eluted DNA
Waste	Tip waste and liquid waste tub

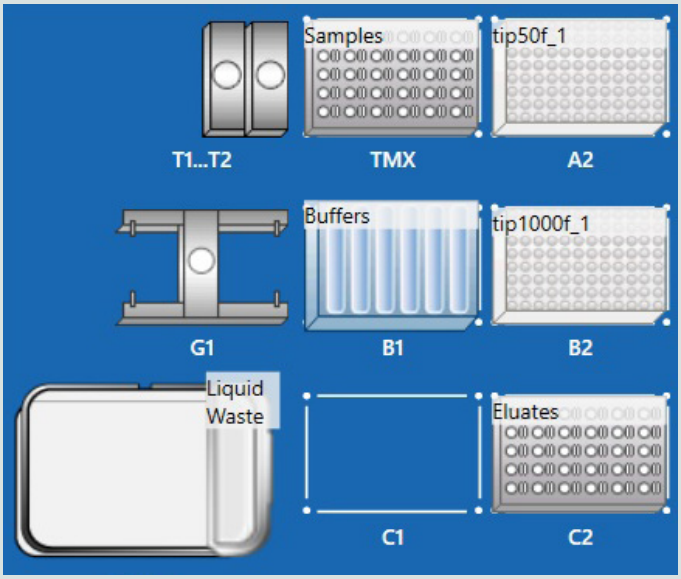

Figure 1: ep*Motion* M5073m worktable layout.

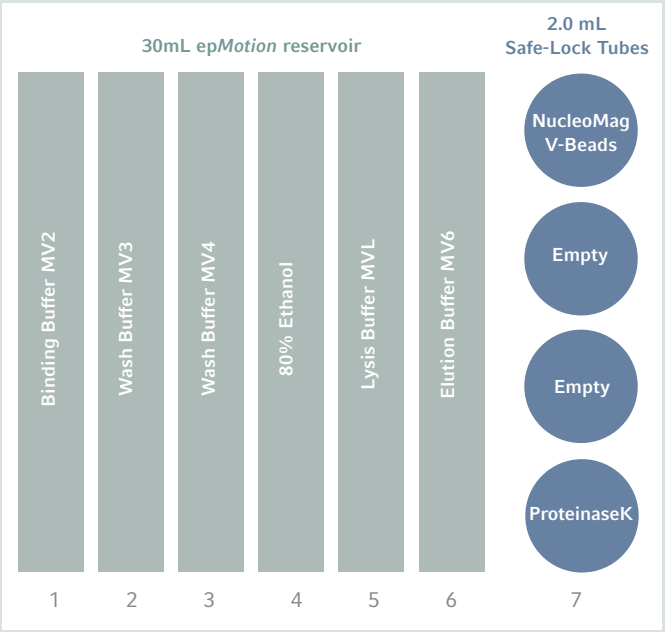

Figure 2: Reservoir rack layout on position B1 of ep*Motion* 5073m worktable.

Results

In order to demonstrate the efficiency of the automated protocol on the ep*Motion* 5073m as described in this publication, samples spiked with viral MS2 phage RNA or T7 phage DNA of different concentrations (n = 6) were processed using the NucleoMag Virus kit from MACHEREY-NAGEL and the following method: "NucleoMag_Virus_5073m.export".
Viral T7 phage DNA was isolated from four different dilutions: 50000 pg, 5000 pg, 5 pg and 0,5 pg. The recovery efficiency was determined by a subsequent qPCR with a Taqman® Probe using the SensiFAST™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System (Fig. 3, dark blue bars).

Viral MS2 phage RNA was isolated from following dilutions 4000 pg, 400 pg, 40 pg and 4 pg in six replicates (n=6). The recovery efficiency was determined by a subsequent qRT-PCR (dark blue bars) with a Taqman® Probe using the SensiFAST™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System (Fig. 4).

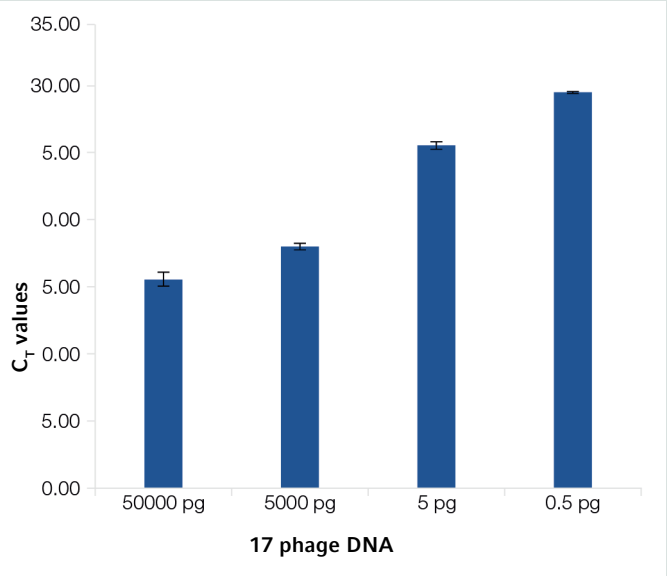

Figure 3: Sensitivity of the viral DNA isolation from plasma samples using T7 phage sDNA.

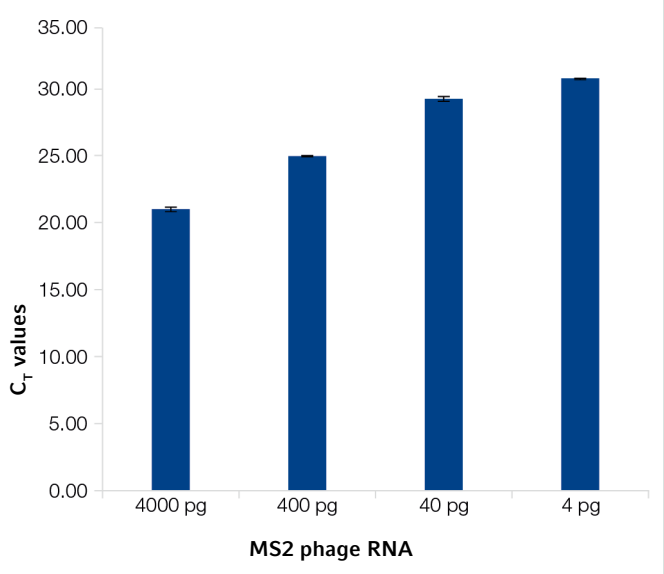

Figure 4: Sensitivity of the viral RNA isolation from plasma samples using MS2 phage RNA.

Ordering information

Description	Order no. International
ep*Motion*® M5073	5073 000.205
TS 50 single channel dispensing tool	5280 000.010
TS 1000 single channel dispensing tool	5280 000.053
PrepRack for 24 Eppendorf Safe-Lock Tubes 2 mL	
Reservoir Rack	5075 754.002
Reservoir rack module TC for 4x Safe-Lock tubes 0.5/1.5/2.0 mL	5075 799.081
Reservoir 30 mL	0030 126.505
Eppendorf Safe-Lock LoBind Tubes, 2.0 mL	0030 120.094
Eppendorf Rack for 24 x Safe Lock 2,0 mL tubes	5075 751.275
epT.I.P.S.® Motion 50 µL Filter	0030 014.413
epT.I.P.S.® Motion SafeRack 1000 µL Filter	0030 014.650
Eppendorf ep*Motion* Tub for liquid waste 400 mL	5075 210.401
NucleoMag® Blood 200 µL 1 x 96 / 4 x 96	744501.1 /.4

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