eppendorf

SHORT PROTOCOL No. 44

Automated gDNA Purification from whole blood samples using the NucleoMag® Blood 200µL kit on the ep*Motion*® 5073m

Introduction

This protocol describes the automated process on the liquid handling system ep*Motion* 5073m using the NucleoMag Blood 200µL Kit from MACHEREY-NAGEL. We show the configuration and pre-programmed method for automated genomic DNA purification from up to 24 fresh human blood samples. The complete procedure according to the user manual of this kit including lysis and Proteinase K treatment is performed automatically on the deck by the ep*Motion* system.

Configuration and method procedure

Method name: NucleoMag_Blood200_5073m.export
This protocol is programmed to process up to 24 samples in parallel on ep*Motion* 5073m. The NucleoMag Blood 200 µL 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.
The blood samples from one blood pool are applied in volumes of 200 µL into 2.0 ml tubes in the PrepRack and placed on the TMX (Fig. 1). To maximize the efficiency of DNA recovery, it is recommended to use Eppendorf DNA LoBind tubes. The required volume 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 B-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 to the samples, followed by an incubation of 10 min at 25°C and simultaneous mixing at 1200 rpm. Afterwards, 300 µL of Binding Buffer MBL2 and 25 µL NucleoMag B-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 B-Beads. After the magnet bead separation, the supernatant is removed and discarded into the liquid waste tub. The genomic DNA attached to the magnetic beads is washed twice with 800 µL of the Wash Buffer MBL3. After second time removing the supernatant, a third washing step follows, using 80% ethanol. The NucleoMag B-Beads are air dried for 7 min at 55°C while mixing at 1200 rpm to remove traces of ethanol.
The genomic DNA is eluted from the NucleoMag B-Beads by adding 105 µL of Elution Buffer MBL5, followed by mixing step at 1300 rpm and 55°C for 5 min. It is possible to adjust the volume of the Elution Buffer MBL5 according to the initial sample amount, to circumvent a strong dilution or concentration of the eluted genomic DNA. After a final magnetic separation step for 2 min, 100 µL supernatant containing the purified genomic DNA is transferred into fresh tubes in the Rack 24 on the C2 position.

MACHEREY-NAGEL

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

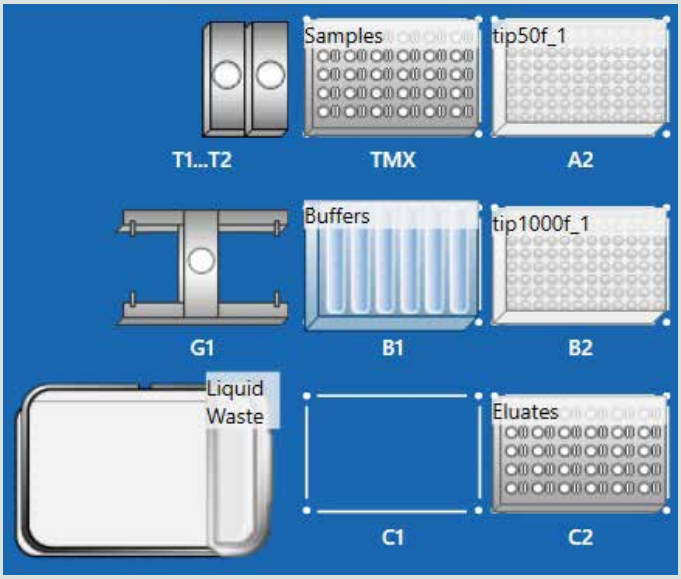

Figure 1: ep*Motion* M5073m worktable layout.

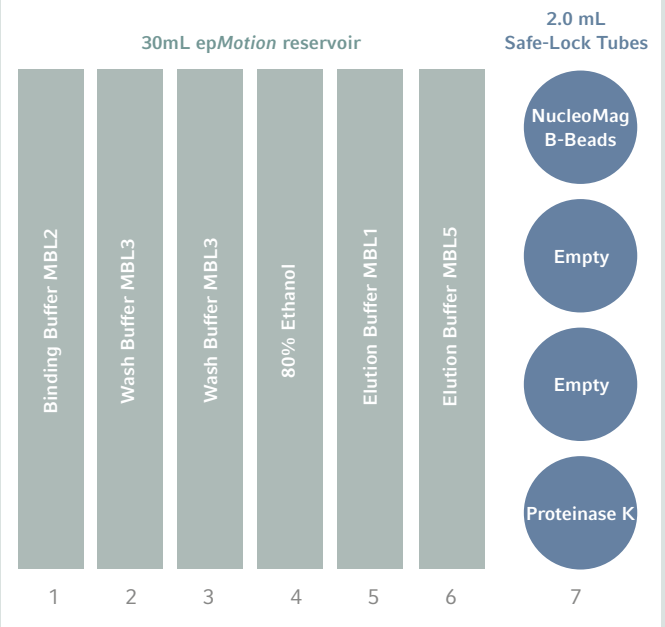

Figure 2: Reservoir rack layout on position B1 of ep*Motion* 5073m worktable.

Results

In order to prove the efficiency of the automated protocol on the ep*Motion* 5073m described in this publication, one human blood pool was split into 12 x 200 µL fractions.

The blood samples were processed by using the following method: NucleoMag_Blood200_5073m.export.

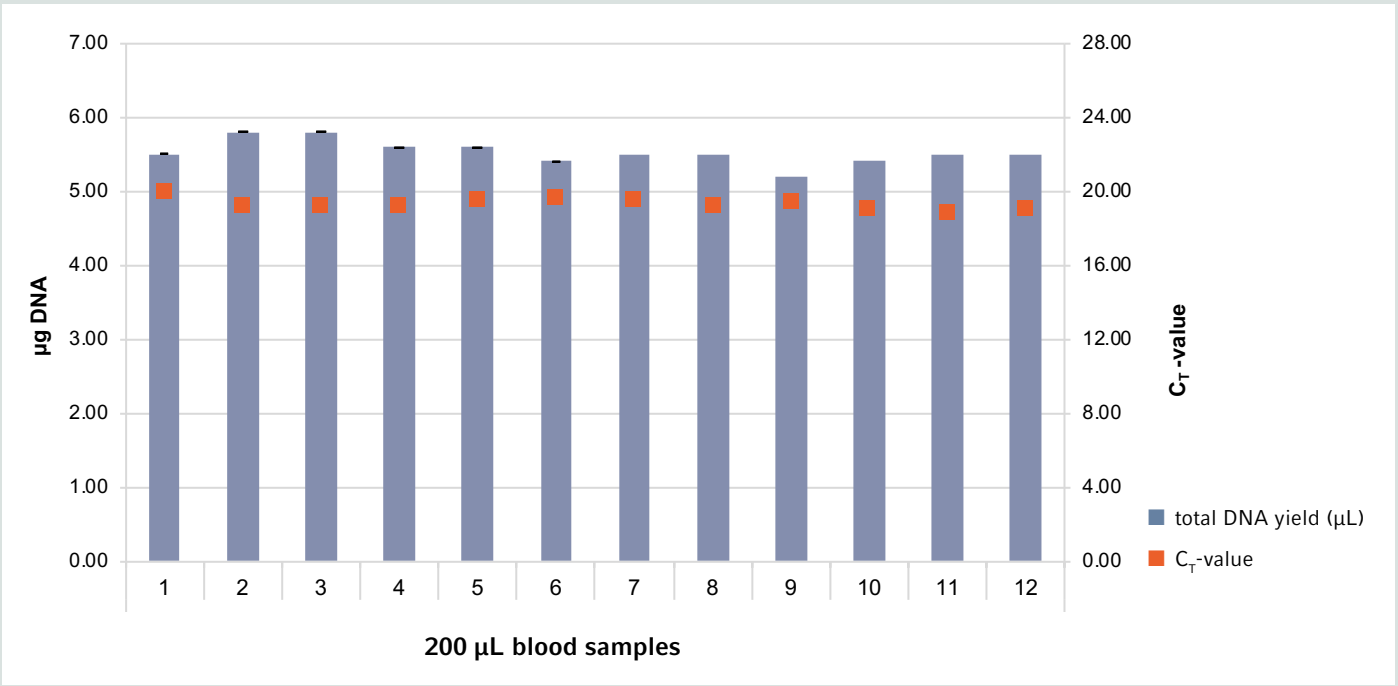

Figure 3: Automated isolation of genomic DNA from fresh human blood samples.

DNA was isolated from fresh 200 µL human blood samples (n = 12) using the NucleoMag Blood 200 µL kit on an ep*Motion* 5073m worktable. The elution of genomic DNA was carried out in 105 µL Elution Buffer MBL5. The DNA concentration of all 12 samples was determined by UV-spectroscopy (Fig. 3, dark blue bars). A subsequent qPCR (orange squares) was performed with a Taqman® Probe for a 250 bp ß-Actin amplicon using the Sensifast™ Probe Lo-ROX kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System (Fig. 3).

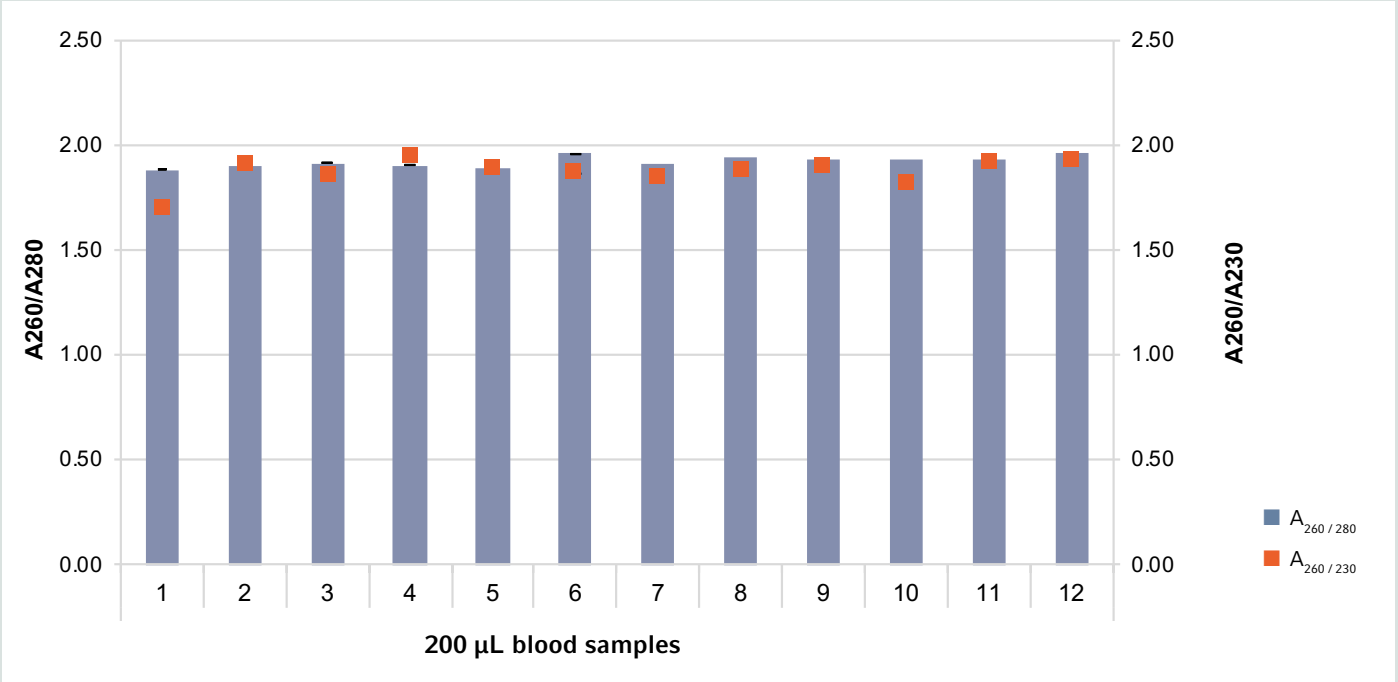

Figure 4: Purity of genomic DNA isolated from fresh human blood samples

DNA was isolated from fresh 200 µL human blood samples (n = 12) using the NucleoMag Blood 200 µL kit on a ep*Motion* 5073m worktable. The total purity was determined by UV-spectroscopy. The ration of A260/280 value is showed in dark blue bars and ration of A260/A230 value in oranges squares.

DNA quality analysis resulted into an average A260/A280 value of 1.92 +/- 0.02 and into an average A260/A230 value of 1.86 +/- 0.06.

Acknowledgement

With gratitude we want to acknowledge MACHEREY&NAGEL for the cooperation in generating results and writing content for this short protocol.

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	5073 751.006
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
ep*Motion*® Tub for liquid waste 400 mL	5075 210.401

MACHEREY&NAGEL	
NucleoMag® Blood 200 µL 1 x 96 / 4 x 96*	744501.1 /.4

*The kit is available at www.mn-net.com

Your local distributor: www.eppendorf.com/contact
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