MACHEREY-NAGEL

NucleoSpin® RNA Blood Midi

NucleoSpin® RNA Blood Midi for RNA isolation from SARSTEDT S-Monovette® RNA Exact

Introduction

Transcriptome analysis of blood samples has an increasing impact. It plays a major role for example in the assessment of the inflammation and inflammation pathways in patients with sepsis. One pitfall is the reliable conservation of the transcriptome after the blood is drawn as any RNA degradation would bias the outcome of the transcriptional analysis.

The S-Monovette® RNA Exact (SARSTEDT) is developed for blood sampling and preserves and stabilizes the RNA and thereby the transcriptome status immediately. Herein we describe the convenient purification of high quality RNA from S-Monovette® RNA Exact with the NucleoSpin® RNA Blood Midi kit.

Products at a glance

NucleoSpin® RNA Blood Midi (MACHEREY-NAGEL)	
Technology	Silica membrane technology
Sample material	400–1300 µL whole blood (fresh or frozen or derived from S-Monovette® RNA Exact Blood Collection Tube for direct blood lysis – no selective erythrocyte lysis)
Typical yield	4–26 µg (1300 µL whole blood) depending on sample quality and leukocyte number
Elution volume	200–400 µL
Binding capacity	700 µg

S-Monovette® RNA Exact (SARSTEDT)	
Tube material	Polypropylene (PP)
Additive volume	7.3 mL
Draw volume	≤ 2.4 mL
Tube size	15 x 100 mm
Storage temperature and stability	Room temperature and stable from –40 °C to +50 °C
Special features	Variable blood draw volume possible

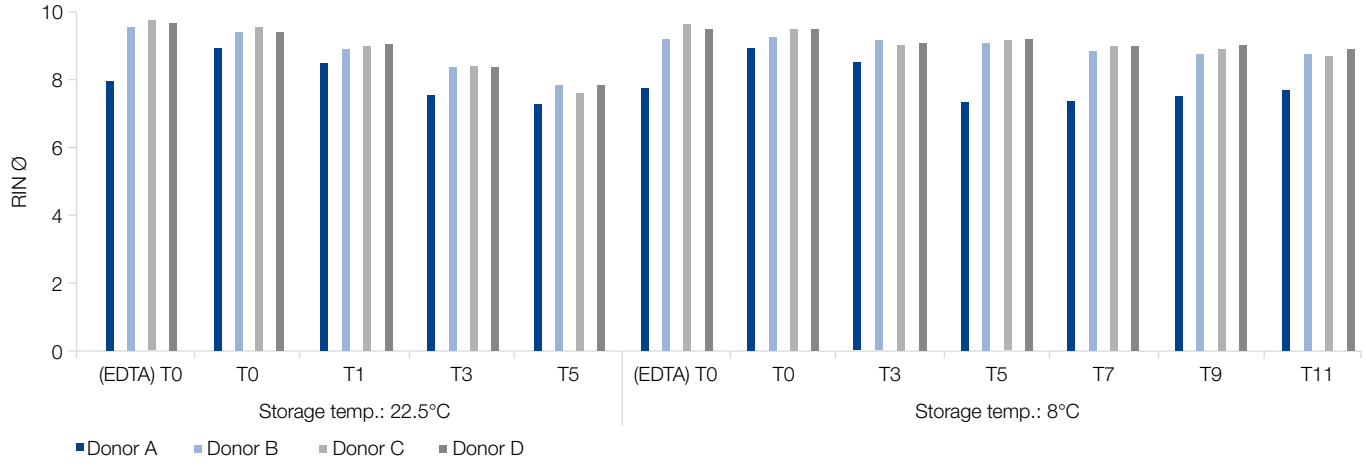
Material and Methods

Blood was drawn from healthy subjects either into S-Monovette® K3E (conventional EDTA blood collection tube) or into a SARSTEDT S-Monovette® RNA Exact. Tubes were inverted according to manufactures instruction in order to ensure proper mixing of blood and reagent within the tube. Tubes were subsequently transported from the site of blood draw to the site of RNA extraction at ambient temperature. RNA was isolated from 2.5 mL of EDTA blood withdrawn from S-Monovette® K3E according to the standard NucleoSpin® RNA Blood Midi kit procedure. Volumes of Lysis Buffer DL and ethanol were proportionally adjusted to the 2.5 mL blood volume processed. Futher, RNA was isolated from the S-Monovette® RNA Exact with NucleoSpin RNA® Blood Midi kit according to a modified protocol (see Table below): Final elution was performed with 200 µL water for all preparations. For a detailed overview of the RNA isolation procedure with the NucleoSpin® RNA Blood Midi from SARSTEDT S-Monovette® RNA Exact see Table below.

SARSTEDT S-Monovette® RNA Exact processing procedure	
1	Provide SARSTEDT S-Monovette® RNA Exact (containing 2.4 mL blood in 7.3 mL stabilizing solution).
2	Add 33 µL Liquid Proteinase K and incubate 15 min at room temperature vigorously shaking the tube. Short spin to clean lid.
3	Apply 4 mL lysate onto the NucleoSpin® RNA Blood Midi Column placed in a Collection Tube (provided). The lysate immediately starts to flow through the column.
4	Allow approx. 1 mL to pass through the NucleoSpin® RNA Blood Midi Column and then apply again 1 mL fresh lysate onto the column. Centrifuge 3 min at 4,500 x *g*.
5	Discard flow through (approx. 5 mL) and Collection Tube. Place NucleoSpin® RNA Blood Midi Column into a new Collection Tube (15 mL, provided).
6	Apply the remaining 4 mL lysate onto the NucleoSpin® RNA Blood Midi Column. Centrifuge 3 min at 4,500 x *g* (no need to discard flow through).
7	Add 1.2 mL MDB onto the NucleoSpin® RNA Blood Midi Column. Centrifuge 3 min at 4,500 x *g*.
8	Discard flow-through and Collection Tube. Place the NucleoSpin® RNA Blood Midi Column into a new Collection Tube (15 mL, provided).
9	Add 240 µL rDNAse onto the NucleoSpin® RNA Blood Midi Column. Incubate 15 min at room temperature.
10	Add 1 mL RB2. Centrifuge 3 min at 4,500 x *g*.
11	Add 3 mL RB3. Centrifuge 3 min at 4,500 x *g*.
12	Discard flow through and Collection Tube. Place the NucleoSpin® RNA Blood Midi Column into a new Collection Tube (15 mL, provided).
13	Add 200 µL H_2O. Centrifuge 3 min at 4,500 x *g*.

Procedure for the RNA isolation from S-Monovette® RNA Exact with the NucleoSpin® RNA Blood Midi kit

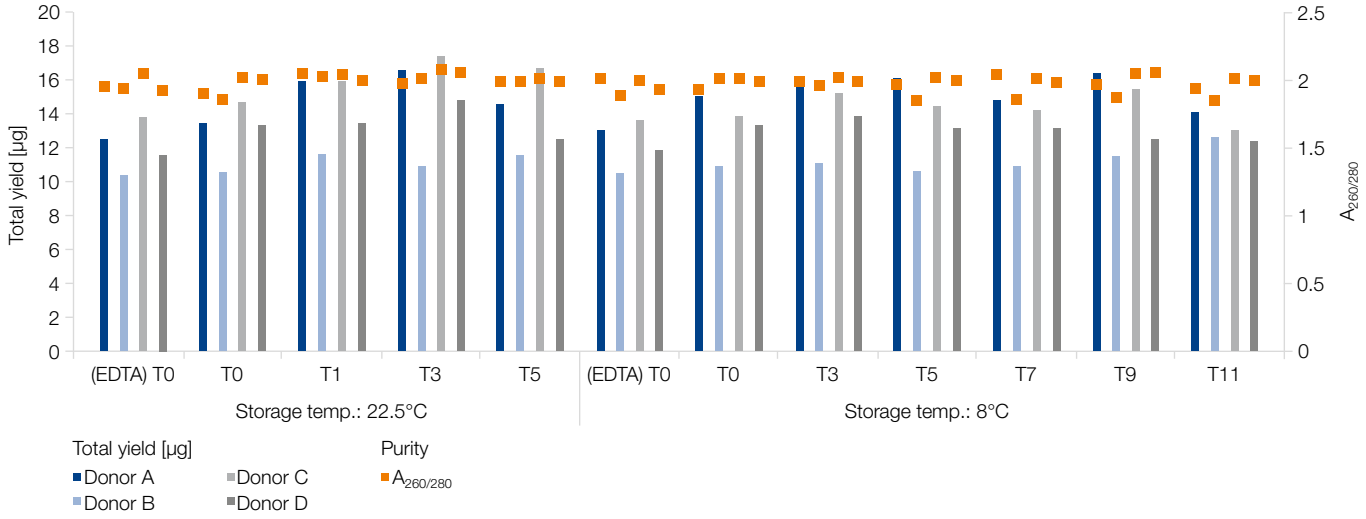
Application data



Quality of isolated RNA with NucleoSpin RNA® Blood Midi from S-Monovette® RNA Exact

After blood collection via S-Monovette® K3E (EDTA) or the S-Monovette® RNA Exact from SARSTEDT, RNA was extracted at different time points (T = days) with the NucleoSpin® RNA Blood Midi kit. Average RIN (Bioanalyzer) of 96 RNA preparation is 8.7 (data not shown). For RNA Excact Tubes, a minimal RIN decrease is observed from day zero to day 5 at 22.5°C storage temperature from 9.3 to 7.7. At 8 °C storage temperature, S-Monovette® RNA Exact yield RNA with RIN from 9.3 at day zero to 8.5 at day 11. RNA quality was further determined with the Fragment Analyzer (Advanced Analytical). The average RNA Quality Number (RQN) over 96 samples is 8.5 (data not shown).Deviation of RIN and RQN is in average 0.7 per sample.

Overall, RIN and RQN correspond well. However, for blood samples collected and stored in EDTA tubes, a considerable decrease of RNA quality and amount during storage for longer than 24 h is well documented in literature (e.g., Pahl and Brune, 2002 in Clinical Chemistry 48:12, 2251–2253; Rainen et al., 2002 in Clinical Chemistry 48:11, 1883–1890). Both publications demonstrate, that a five day incubation of EDTA blood at room temperature causes approximately 75 % loss of RNA, and a severe loss of RNA integrity. Thus, SARSTEDT's S-Monovette® RNA Exact is well suited for the preservation of RNA quality within whole blood samples and NucleoSpin® RNA Blood Midi enables a convenient and efficient RNA isolation from such tubes.



Determination of RNA yield and purity after extraction from S-Monovette® RNA Exact with the NucleoSpin® RNA Blood Midi kit

After blood collection via S-Monovette® K3E (EDTA) or the S-Monovette® RNA Exact, RNA was extracted at different time points (T = days) with the NucleoSpin® RNA Blood Midi kit. The average RNA yield from 2.5 mL whole blood was determined by UV spectrometry (blue bars) and showed an average yield of 13 µg for 96 analyzed samples, ranging from 10–17 µg per sample (data not shown). The average RNA yield (Donor A: 15 µg; Donor B: 11 µg; Donor C: 15 µg; Donor D: 13 µg) for each single donor was determined and yield deviation between two duplicate preparations was 8 %, ranging from 0 % to 27 % deviation, whereas 75 % of the duplicate preparations showed less than 11 % deviation. RNA purity was determined by measurement of the A_{260}/A_{280} ratio (orange squares). Ratio A_{260}/A_{280} values obtained were in average 2.0 ranging from 1.8 to 2.1 indicating high RNA purity. There were identical purities obtained for EDTA and S-Monovette® RNA Exact. Furthermore, 600 µL (single column loading) and 1200 µL (double column loading) lysate from S-Monovette® RNA Exact were applied after the Proteinase K digestion step onto NucleoSpin® RNA Blood mini spin column and further processed according to the standard procedure of the NucleoSpin RNA® Blood kit (REF 740200). One to two microgramm of RNA with RIN values equivalent to the midi column procedure were obtained (data not shown).

Conclusion

The NucleoSpin® RNA Blood Midi kit enables convenient and efficient isolation of RNA from S-Monovette® RNA Exact. Even after storage at cool (8 °C) and ambient (22.5 °C) temperature for up to five days after blood collection, RNA of high quality and yield can be isolated. RNA Integrity Numbers (RIN) of > 8 / > 7 are obtained and RNA concentrations is 49–87 ng/µL within the eluates. Storage at 8 °C for 11 days still yields high quality RNA with RIN > 8, without any decrease of RNA yield.

Ordering information

Product	Specifications	Preps	REF
NucleoSpin® RNA Blood Midi	Kit based on silica membrane technology to isolate RNA from fresh and frozen blood with direct blood lysis – sufficient for 20 preps with 2.5 mL blood in SARSTEDT S-Monovette® RNA Exact tubes.	20	740210.20
NucleoSpin® RNA Blood	Mini spin kit for on silica membrane technology to isolate RNA from fresh and frozen blood with direct blood lysis – sufficient for 10 / 50 preps with 0.6–1.2 mL blood in SARSTEDT S-Monovette® RNA Exact tubes.	10 / 50	740200.10 / .50
SARSTEDT S-Monovette® RNA Exact	See *www.SARSTEDT.com*	20 / 80	01.2048.001