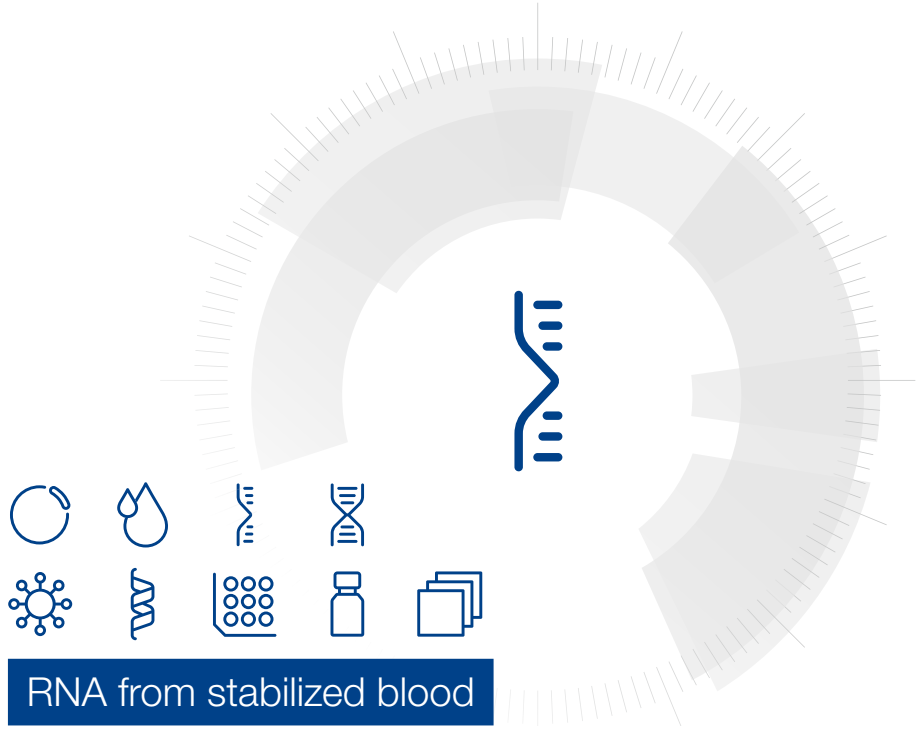
Bioanalysis

MACHEREY-NAGEL

User manual



RNA from stabilized blood

■ NucleoSpin® Dx RNA Blood

IVD *In-Vitro* Diagnostic Medical Device

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CE

REF 740201.50

Σ 50 preps

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1 Components

1.1 Kit contents

	NucleoSpin® Dx RNA Blood	
REF	**Symbol**	**50 preps 740201.50**
Wash Buffer RB2	BUF RB2	13 mL
Wash Buffer RB3 (Concentrate)**	BUF RB3 Conc.	12 mL
Membrane Desalting Buffer MDB	BUF MDB	25 mL
Reaction Buffer for rDNase	BUF Reaction Buffer	7 mL
rDNase, RNase-free (lyophilized)*	rDNase	2 vials (size D)
Liquid Proteinase K	Liquid Proteinase K	600 µL
RNase-free H_2O	H_2O	13 mL
NucleoSpin® RNA Blood Columns (light blue rings -plus Collection Tubes)	RNA Blood Column	50
Lysis Tubes (2 mL, with lid)	Lysis Tubes	50
Elutions Tubes (1.5 mL)	Elution Tubes	50
Collection Tubes (2 mL)	Collection Tubes	150
User manual		1

* For preparation of working solutions and storage conditions see section 3.

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol (to prepare Wash Buffer RB3)

Consumables

- Sterile RNase-free tips

Equipment

- Manual pipettors
- Vortex mixer
- Centrifuge for 2 mL microcentrifuge tubes
- Personal protection equipment (e.g., lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended to read the detailed protocol sections of this user manual. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

MACHEREY-NAGEL user manuals are available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

Contact information

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Benutzerhandbücher in weiteren Sprachen sind im Download-Bereich auf der Produktseite verfügbar.

Les manuels d'utilisation dans d'autres langues sont disponibles dans la section Téléchargements de la page du produit.

Los manuales de usuario en otros idiomas están disponibles en la sección de descargas de la pagina del producto.

2 Product description

2.1 The Intended purpose

NucleoSpin® Dx RNA Blood is a kit for the isolation of human RNA from whole blood collected in SARSTEDT's *S-Monovette® RNA Exact* for the subsequent *in-vitro* diagnostic analysis. The product provides purified human RNA to be used for subsequent down-stream analysis such as RT-PCR, qRT-PCR or RNA-Seq to obtain information about the RNA expression level in the sample. The product is used by professional users in diagnostic laboratories.

The **NucleoSpin® Dx RNA Blood** kit is not suitable for self-testing or near-patient testing. The user should have experience with molecular biological techniques including experience with whole blood and other potentially infectious human sample materials.

The use of suitable controls is recommended.

Only blood from the *S-Monovette® RNA Exact* can be used.

The kit is for manual use.

2.2 Product use limitations

The **NucleoSpin® Dx RNA Blood** kit is suitable for the purification of RNA from blood collected in the *S-Monovette® RNA Exact*. The N**ucleoSpin® Dx RNA Blood** has not been validated for other sample materials (i.e. EDTA blood).

Please note that a potential inhibitory effect by substances in the blood (e.g., pharmaceuticals) cannot be completely excluded. We therefore recommend the use of appropriate controls.

2.3 Quality control

In accordance with MACHEREY-NAGEL's Quality Management System, each lot of NucleoSpin® Dx RNA Blood kit is tested against predetermined specifications to ensure consistent product quality.

2.4 Introduction and kit specifications

The **NucleoSpin® Dx RNA Blood** kit offers RNA isolation from whole blood collected in Sarstedt *S-Monovette® RNA Exact* blood collection tubes. One of the most important aspects in RNA purification is to prevent changes in the expression level of transcripts after blood draw and before blood lysis and to prevent RNA degradation during storage, transportation and isolation. With the **NucleoSpin® Dx RNA Blood** method, RNA is isolated from blood collected in *S-Monovette® RNA Exact* in which leukocytes (the main source of RNA in whole blood) and other blood cells, are lysed immediately upon contact of the blood with the stabilization solution present in the blood collection tube. This stabilization solution within the *S-Monovette® RNA Exact* immediately inactivates RNases (which are present in virtually all biological materials), facilitates storage and transport of the blood samples and creates appropriate binding conditions that favor adsorption of RNA to the silica membrane. Contaminating DNA, which is also bound to the silica membrane, is removed by a recombinant DNase solution (supplied) which is directly applied onto the silica membrane during the preparation. Simple washing steps with a chaotropic wash buffer (RB2) and an ethanolic wash buffer (RB3) remove salts, metabolites, and macromolecular cellular components. Pure RNA is finally eluted under low ionic strength conditions with RNase-free H_2O (supplied).

The RNA preparation using **NucleoSpin® Dx RNA Blood** kits is performed at room temperature. A refrigerated centrifuge is not necessary. The eluate, however, should be handled with care

because RNA is very sensitive to trace contaminations of RNases, often found on general lab ware, fingerprints, and dust. To ensure RNA stability, keep RNA frozen at -20 °C for short-term or -70 °C for long-term storage.

- **NucleoSpin® Dx RNA Blood** kit is recommended for the isolation of RNA from whole blood colleced in Sarstedt *S-Monovette® RNA Exact* blood collection tubes. It is not to be used for the isolation of RNA from blood collected in other blood collection tubes like EDTA, citrate, or heparin containing tubes.
- The **NucleoSpin® Dx RNA Blood** kits allow the purification of RNA with an A_{260}/A_{280} ratio typically within the range of 1.9 – 2.1 (measured in TE buffer, pH 7.5).
- The isolated RNA is ready to be used for subsequent down-stream analysis such as RT-PCR, qRT-PCR or RNA-Seq to obtain information about the RNA expression level in the sample.
- RNA isolated with the NucleoSpin® Dx RNA Blood kits is typically of high integrity. However, RNA integrity strongly depends on the sample quality which is influenced by storage temperature and duration.

The amount of DNA contamination is significantly reduced during on-column digestion with rDNase. However, in very sensitive applications, it may be possible to detect traces of DNA. The probability of DNA detection with PCR increases with:

1. the number of DNA copies per preparation: single copy target < plastidial / mitochondrial target < plasmid transfected into cells.
2. decreasing PCR amplicon size.

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Dx RNA Blood
Sample material	1.2 mL solution of stabilized blood from *S-Monovette® RNA Exact* blood collection tubes (SARSTEDT REF 01.2048.001).
Format	Mini spin column
Fragment size	> 200 nt
Typical yield	> 1 µg (0.7 – 4.2 µg) per preparation from blood from healthy subjects
A_{260}/A_{280}	1.6 – 2.2 (typically 1.9 – 2.1)
Elution volume	60 µL or 40 µL
Theoretical binding capacity	200 µg
Preparation time	55 min / 6 preps

The **NucleoSpin® Dx RNA Blood** kit contains one protocol that allows the use of 1.2 mL solution of stabilized blood from Sarstedt *S-Monovette® RNA Exact*.

The isolated RNA can be used as a template in (q)RT-PCR-reactions and RNA-Seq analysis. Generally, a volume of 1 – 4 µL of a 60 µL eluate from one preparation is suitable as a template for RT-PCR.

2.5 Analytical and clinical performance

Analytical performance of **NucleoSpin® Dx RNA Blood** was evaluated with down-stream quantification of isolated RNA.

Within run repeatability regarding RNA yield was determined from 12 independent preparation runs of 6 preparations each, for which RNA was quantified spectrophotometrically and / or fluriometrically. The average coefficient of variation (CV) of RNA yield was 11 % (6 – 20 %) within a run.

Between run variation regarding RNA yield was determined by comparison of RNA yield from two runs, each comprising 6 preparations. The average RNA yield of the two sets differed by 2 %.

Batch-to-batch repeatability was determined by comparison of RNA yield from three batches with six preparations per batch. Average yield among the batches differed by 1 – 3 %.

Reproducibility between operators was determined by comparison of RNA yield obtained by two operators with six preparations per operator. The average RNA yield between the operators differed by 35 %.

In a study focusing on deficiencies in cellular stress response, differential splicing of the XBP1 gene transcripts was investigated. RNA was isolated from human blood collected in the SARSTEDT's Monovette® RNA Exact. From 3 donors, two blood samples were taken in close succession and subsequently stored for either 2 hours or 24 hours prior to RNA extraction using the **NucleoSpin® Dx RNA Blood** kit. RNA quantity and quality was determined followed by qRT-PCR analyis of four transcripts (two housekeeping genes (ACTB, HPRT) and two splicing variants of XBP1).

Table 2: RNA yield and quality after RNA isolation from SARSTEDT Monovette® RNA Exact. 1, 2, 3 represent the donors; A, B represent duplicates from 2 blood collection tubes. RNA was isolated after 2 h (I) and 24 h (II) post blood draw. RNA was analysed with NanoDrop™ and Qubit instruments.

Sample	Nanodrop			Qubit
	ng / µL	260 / 280	260 / 230	ng / µl
1A - I	45.2	2.08	1.94	44.9
1A - II	46.5	2.08	1.35	46.7
2A - I	50.4	2.05	1.77	47.2
2A - II	52.0	2.06	1.04	52.9
3A - I	55.3	2.02	1.31	52.4
3A - II	54.4	2.03	1.68	50.8
1B - I	44.0	2.03	1.66	41.4
1B - II	42.6	2.07	1.63	40.1
2B - I	53.0	2.00	1.06	49.9
2B - II	50.9	2.04	1.72	50.8
3B - I	50.3	2.04	1.20	50.1
3B - II	54.1	2.02	1.73	52.6
Ø	**49.9**	**2.0**	**1.5**	**48.3**
Total ave. RNA yield (40 µL elution volume)	**2.5 µg**			**2.4 µg**

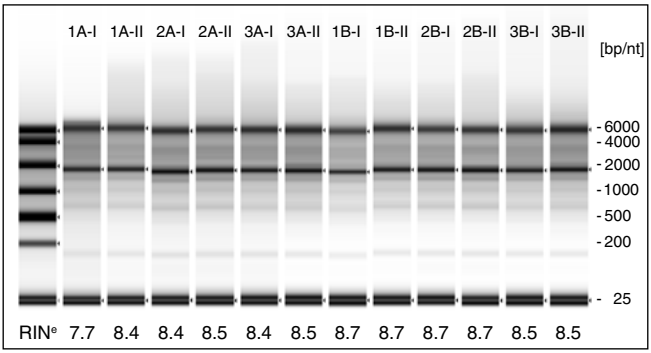

Figure 1 RNA integrity determination (RIN^e = RNA Integrity Number via RNA ScreenTape®). An average RIN^e of 8.5 was obtained. Samples from left to right represent samples of Table 2 (1A-I to 3B-II).

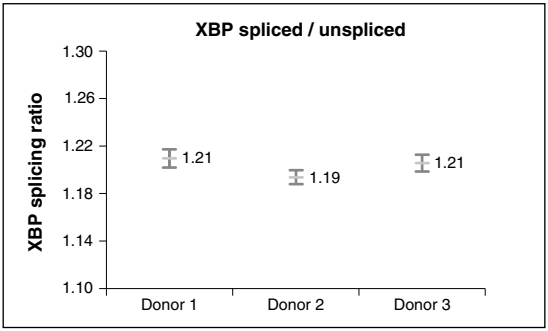

Figure 2 Analysis of XBP1 splicing variants (spliced/unspliced) via qRT-PCR from three healthy donors.

Conclusions

A total of twelve RNA extractions were conducted using Monovette® RNA Exact blood collection tubes and NucleoSpin® Dx RNA Blood kit. All samples yielded RNA suitable for subsequent qRT-PCR analysis.

A comparison of the ratio of spliced to unspliced RNA transcripts derived from the XBP1 gene revealed minor differences between the three individual donors, as visualized in the figure above.

The illustrated standard deviations per donor, represented by the grey bars, encompass technical replicates at two levels: first, blood collection with consecutive draws, and second, storage time (2 and 24 hours) of the blood collection tube. The analytical results emphasize the ability to detect slight differences in cellular stress levels even within the cohort of three healthy donors.

Existing literature highlights that patients exhibiting aberrant cellular stress responses tend to manifest spliced/unspliced ratios ranging from 0.5 to 1.5. Given this context, it is evident that the processes of blood collection, storage, and RNA isolation demonstrate robustness and precision sufficient for the scrutiny of individuals afflicted by cellular stress response disorders.

In-vitro diagnostic use of NucleoSpin® RNA Blood kits in combination with *S-Monovette® RNA Exact* is exemplified in the following publications

- Linden J *et al.* (2020): Impact of RNA Stabilizing Blood Collection Tubes on Gene Expression Data Validity – A Comparison of S-Monovette® RNA Exact, PAXgene™ Blood RNA Tubes & Tempus™ Blood RNA Tubes. https://www.sarstedt.com/fileadmin/user_upload/Mediacenter/Studien/an_007_rna-exact_monovette_0123.pdf
- Reith M. *et al.* (2022): Novel, Apparently Silent Variant in MFSD8 Causes Neuronal Ceroid Lipofuscinosis with Marked Intrafamilial Variability. Int. J. Mol. Sci. 2022, 23, 2271. https://doi.org / 10.3390/ijms23042271.

2.6 Handling, preparation, and storage of starting materials

NucleoSpin® Dx RNA Blood kit is designed for isolation of total RNA from blood collected in Sarstedt *S-Monovette® RNA Exact*.

Blood has to be collected in the Sarstedt *S-Monovette® RNA Exact* according to the instruction manual for the *S-Monovette® RNA Exact.* Recommended transport and storage conditions of the *S-Monovette® RNA Exact* have to be followed for blood collected within these tubes. The RNA stabilizing efficiency of the *S-Monovette® RNA Exac*t is validated for 5 days at 22 °C and 14 days at 8 °C. For long term storage freezing at below -40 °C is possible; a long term storage temperature at -80 °C is recommended.

For details see: https://www.sarstedt.com/produkte/diagnostik/venenblut/s-monovette/produkt/01.2048.001/

Wear gloves at all times during the preparation. Change gloves frequently.

2.7 Elution procedures

It is possible to adjust the elution volume from 60 µL (standard elution volume) to 40 µL, resulting in a slightly higher RNA concentration.

Eluted RNA should be immediately placed and kept on ice for optimal stability and to prohibit omnipresent RNases (general lab ware, fingerprints, dust) from degrading the RNA. For short term storage, freeze at -20 °C, for long term storage, freeze at -70 °C or below.

3 Storage conditions and preparation of working solutions

Attention: *Buffers RB2, and MDB contain chaotropic salts. Wear gloves and goggles!*

CAUTION: Buffers RB2, and MDB contain guanidinium salts which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Attention:

- Check all components for damages after receiving the kit. If kit contents, like buffer bottles or blister packages are damaged, contact MACHEREY-NAGEL technical support and customer service, or your local distributor.
- Do not use damaged kit components.
- The lyophilized rDNase is shipped at ambient temperature within the kit. Store lyophilized rDNAse (RNase-free) at 4 °C on arrival (stable until: see package label).
- All other kit components should be stored at 15 – 25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts.
- **NucleoSpin® RNA Blood Columns** can be used until the expiration date specified on the kit box
- After first use, store Liquid Proteinase K at 4 °C or -20 °C.
- Check that 96 – 100 % ethanol is available as additional solution to prepare Wash Buffer RB3.

Before starting any **NucleoSpin® Dx RNA Blood** protocol, prepare the following:

- **rDNase (RNase-free)**: Add indicated volume of Reaction Buffer for rDNase (see table below) to the rDNase vial and incubate for 1 min at room temperature. Gently swirl the vials to completely dissolve the rDNase. Be careful not to mix rDNase vigorously as rDNase is sensitive to mechanical agitation. Dispense into aliquots and store at -20 °C. The frozen working solution is stable for 6 months. Do not freeze / thaw the aliquots more than three times. (Be careful when opening the vial as some particles of the lyophilisate may be attached to the lid.)
- **Wash Buffer RB3**: Add the indicated volume of 96 – 100 % ethanol (see table below) to Buffer RB3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer RB3 at 15 – 25 °C for up to one year.

	NucleoSpin® Dx RNA Blood
REF	**50 preps** **740201.50**
Wash Buffer RB3 Concentrate	12 mL Add 48 mL ethanol
rDNase, RNase-free (lyophilized)	2 vials (size D) Add 2.5 mL Reaction Buffer for rDNase to each vial

4 Safety instructions

When working with the **NucleoSpin® Dx RNA Blood** kits wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***)

The waste generated with the **NucleoSpin® Dx RNA Blood** kits has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing stabilization reagent within the *S-Monovette® RNA Exact* and Proteinase K treatment but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 RNA isolation from SARSTEDT *S-Monovette*® *RNA Exact* with NucleoSpin® Dx RNA Blood

The procedure below provides instructions for processing a single blood sample. However, several sample can be processed at the same time; the number depends on the capacity of the mirocentrifuge used.

Before starting the preparation:

- Check if Wash Buffer RB3 was prepared according to section 3.
- Check if rDNase was prepared according to section 3.
- The complete procedure should be performed at room temperature (15 – 25 °C).
- Generally, do not mix reagents and columns from different kits and lots.

5.1 Protocol at a glance

Supplemental protocol-overview: Carefully read the detailed protocol (section 5.2) before starting the procedure.

Lyse blood	**1**	Provide blood containing *S-Monovette® RNA Exact*
	2	Transfer 1.2 mL solution into a 2 mL tube.
	3	10 µL Proteinase K
	4	RT, 15 min (shaking)
	5	Short spin to clean lid
Bind nucleic acid	**6**	Load 600 µL lysate onto column
	7	11,000 x *g*, 30 s
	8	Load remaining lysate (~600 µL)
	9	11,000 x *g*, 30 s
Desalt silica membrane	**10**	350 µL MDB
	11	11,000 x *g*, 30 s
Digest DNA	**12**	95 µL rDNase
	13	RT, 15 min
Wash silica membrane	**14**	200 µL RB2
	15	11,000 x *g*, 30 s
	16	600 µL RB3
	17	11,000 x *g*, 30 s
	18	250 µL RB3
	19	11,000 x *g*, 2 min
Elute RNA	**20**	Place column into fresh Collection Tube (1.5 mL)
	21	60 µL RNase-free H_2O
	22	11,000 x *g*, 30 s

5.2 Procedure in detail

1	Provide SARSTEDT *S-Monovette® RNA Exact* (containing approx. 2.4 mL blood in 7.3 mL stabilizing solution).
2	**Transfer 1.2 mL** solution (whole blood collected in *S-Monovette® RNA Exact)* from the *S-Monovette® RNA Exact* and transfer into a Lysis Tube (2 mL tube with lid, provided).
3	Add **10 µL Liquid Proteinase K**.
4	Incubate **15 min** at **room temperature** vigorously shaking the tube. Alternatively vortex vigorously for 30 sec. before incubation without shaking.
5	Short spin to clean lid.
6	Apply **600 µL** lysate onto the **NucleoSpin® RNA Blood column** placed in a Collection Tube (provided). The lysate might start to flow through the column – this is o.k. *Note: Do not pipette more than 650 µL into the spin column, this will cause the column to overflow! Avoid formation of foam and aerosols! Avoid wetting the rim (edge) of the column.*
7	Centrifuge **30 s** at **11,000 x *g***. Discard flow-through and Collection Tube. Place the column in a new Collection Tube (2 mL; provided).
8	**Apply the remaining lysate** (approx. 600 µL) onto the NucleoSpin® RNA Blood Mini column.
9	Centrifuge **30 s** at **11,000 x *g***. Discard flow-through and Collection Tube. Place the column in a new Collection Tube (2 mL; provided).
10	Add **350 µL MDB** (Membrane Desalting Buffer) onto the column.
11	Centrifuge **30 s** at **11,000 x *g***. *Note: After centrifugation, the column can remain in the Collection Tube including the flow-through! The flow-through may be slightly brown. The flow-through can remain in the tube without disturbing DNA digestion.*
12	Add **95 µL rDNase** onto the column.
13	Incubate at **room temperature** for **15 min**. *Note: Centrifugation after incubation is not necessary.*
14	Add **200 µL Buffer RB2** to the NucleoSpin® RNA Blood Column. *Note: Buffer RB2 will inactivate the rDNase.*
15	Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and Collection Tube and place the column into a new Collection Tube (2 mL; provided).

16	Add **600 µL Buffer RB3** to the NucleoSpin® RNA Blood Column. *Note: Make sure that residual buffer from the previous steps is washed away with Buffer RB3, especially if the lysate has been in contact with the inner rim of the column during loading of the lysate onto the column. For efficient washing of the inner rim flush it with Buffer RB3.*
17	Centrifuge for **30 s** at **11,000 x *g***. Discard flow-through and place the column into a new Collection Tube (2 mL; provided).
18	Add **250 µL Buffer RB3** to the NucleoSpin® RNA Blood Column.
19	Centrifuge for **2 min** at **11,000 x *g***. In this step, ethanol is removed from the column. *If for any reason the liquid level in the Collection Tube has reached the NucleoSpin® RNA Blood Column after centrifugation, discard flow-through, and centrifuge again.*
20	Place the column into a nuclease-free Collection Tube (1.5 mL, supplied) and discard the Collection tube with flow-through from the previous step.
21	Add **60 µL RNase-free H_2O** (supplied) onto the column. *Note: Alternatively, elution can be performed with 40 µL.*
22	Centrifuge **30 s** at **11,000 x *g***. The RNA is eluted into the Collection Tube.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
RNA is degraded / no RNA obtained	*RNase contamination* • Create an RNase-free working environment. Wear gloves during all steps of the procedure. Change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250 °C before use.
Poor RNA quality or yield	*Reagents not applied or restored properly* • Reagents not properly restored. Add the indicated volume of ethanol to Buffer RB3 Concentrate and mix. Reconstitute and store lyophilized rDNase according to instructions given in section 3. • Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added. *Kit storage* • Store lyophilized / reconstituted rDNase according to instructions given in section3. • Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation. • Keep bottles tightly closed in order to prevent evaporation or contamination. *Ionic strength and pH influence A_{260} absorption as well as ratio A_{260}/A_{280}* • For adsorption measurement of isolated RNA, use 5 mM Tris pH 8.5 as diluent. Please see also: -Manchester, K L. 1995. Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. Biotechniques 19, 208 – 209. -Wilfinger, W W, Mackey, K and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 22, 474 – 481.
Clogged NucleoSpin® Column / Poor RNA quality or yield	*Sample material* • Bad sample quality. Make sure blood is collected into SARSTEDT *S-Monovette® RNA Exact* according to the instruction for use. Make sure to mix the blood with the stabilization solution within the *S-Monovette® RNA Exact immediately upon withdrawel of the blood from the donor* according to the instruction for use. *Inappropriate lysis / binding conditions* • Make sure to shake during lysis incubation according to one of the two alternatives decribed in section 5.2.4. – shaking is important for the procedure!

Problem	Possible cause and suggestions
Contamination of RNA with genomic DNA	*rDNase not active* • Reconstitute and store lyophilized rDNase according to instructions given in section 3. *DNase solution not properly applied* • Pipette rDNase solution directly onto the center of the silica membrane. *High leukocyte number* • The higher the leukocyte number, the higher the risk to detect residual DNA in the eluted RNA. To avoid this, follow one of the recommendations below. *DNA detection system too sensitive* • The amount of DNA contamination is effectively reduced during the on-column digestion with rDNase. However, it cannot be guaranteed that the purified RNA is 100 % free of DNA. Therefore, in very sensitive applications it might still be possible to detect DNA. • The probability of DNA detection with PCR increases with: - the number of DNA copies per preparation: single copy target < mitochondrial target < plasmid transfected into cells - decreasing of PCR amplicon size. • Use larger PCR targets (e.g., > 500 bp) or intron spanning primers if possible.
Suboptimal performance of RNA in downstream experiments	*Carry-over of ethanol or salt* • Do not let the flow-through touch the column outlet after the second Buffer RB3 wash. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic Buffer RB3 completely. • Check if Buffer RB3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Buffer RB3. *Store isolated RNA properly* • Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases (general lab ware, fingerprints, dust) will degrade the isolated RNA. For short term storage freeze at -20 °C, for long term storage freeze at -70 °C.

Please contact:
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e-mail: support@mn-net.com

6.2 Notification requirement

Please note that any serious incident that has occurred in relation to the product shall be reported immediately to the manufacturer and the competent authority of the European member state in which the incident occurred. European vigilance contact points: https://ec.europa.eu/health/md_sector/contact_en

6.3 General Literature

Ceylan A et al. (2022): Evaluation of mRNA Expression Levels of IL-10, IL-12, TGF-β, FOXP3, IFN in Multiple Sclerosis Patients. Eastern Anatolian Journal of Science Volume VIII, Issue I, 2022, 9 – 14.

Genre F et al (2020): Omentin: a biomarker of cardiovascular risk in individuals with axial spondyloarthritis. Scientific Reports 10:9636, https://doi.org/10.1038/s41598-020-66816-x

Jennings LJ (2022): Normalization of NPM1 mutant transcript to thewild-type transcript. eJHaem. 2022;3:1343 – 1345.

Pulito-Cueto, V. et al. (2022): Angiogenic T Cells: Potential Biomarkers for the Early Diagnosis of Interstitial Lung Disease in Autoimmune Diseases?. Biomed-icines 2022, 10, 851. https://doi.org/10.3390/biomedicines10040851.

Shimizu T et al. (2022): Depletion of R270C Mutant p53 in Osteosarcoma Atten-uates Cell Growth but Does Not Prevent Invasion and Metastasis In Vivo. Cells. 2022, 11, 3614. https://doi.org/10.3390/cells11223614.

Van der Sijde (2020): RNA from stabilized whole blood enables more compre-hensive immune gene expression profiling compared to RNA from peripheral blood mononuclear cells. PLoS ONE 15(6): e0235413. https://doi.org/10.1371/journal.pone.0235413

Yamagata H et al. (2023): Interferon signaling and hypercytokinemia-related gene expression in the blood of antidepressant non-responders. Heliyon 9 (2023) e13059.

Yuksel F et al (2023): The phenotypic spectrum of pathogenic ATP1A1 variants expands: the novel p.P600R substitution causes demyelinating Char-cot–Marie–Tooth disease. Journal of Neurology, https://doi.org/10.1007/s00415-023-11581-w

6.4 Ordering information

Product	REF	Pack of
CE-IVD marked kits		
NucleoSpin® Dx RNA Blood	740201.50	50
NucleoSpin® Dx Blood	740899.50 / .250	50 / 250
NucleoSpin® Dx Virus	740895.50	50
NucleoSpin® Dx Pathogen	744215.4	4 × 96
Kits for research purposes		
NucleoSpin® RNA Blood	740200.10 / 50	10 / 50
NucleoSpin® RNA Blood Midi	740210.20	20
NucleoSpin® 8 RNA Blood	740220 / .5	12 × 8 / 60 × 8
NucleoSpin® 96 RNA Blood	740225.2 / .4	2 × 96 / 4 × 96

Visit *www.mn-net.com* for more detailed product information.

6.5 Explanation of symbols

REF Item number

LOT Batch identification

Manufacturer

IVD *In-vitro* diagnostic products

Please read instructions for use

Sufficient for < n> tests

Permitted storage temperature range

Use by

Caution: Further information in user manual

Do not reuse

6.6 Product use restriction / warranty

NucleoSpin® Dx RNA Blood kit components are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

ALL OTHER PRODUCTS NOT LABELED AS IVD ARE NOT SUITED FOR ANY CLINICAL USE (INCLUDING, BUT NOT LIMITED TO DIAGNOSTIC, THERAPEUTIC AND/OR PROGNOSTIC USE).

No claim or representations is intended for its use to identify any specific organism or for clinical use (included, but not limited to diagnostic, prognostic, therapeutic, or blood banking). It is rather in the responsibility of the user or -in any case of resale of the products -in the responsibility of the reseller to inspect and assure the use of the DNA/RNA/protein purification products of MACHEREY-NAGEL for a well-defined and specific application.

MACHEREY-NAGEL shall only be responsible for the product specifications and the performance range of MN products according to the specifications of in-house quality control, product documentation and marketing material.

This MACHEREY-NAGEL product is shipped with documentation stating specifications and other technical information. MACHEREY-NAGEL warrants to meet the stated specifications. MACHEREY-NAGEL's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Supplementary reference is made to the general business terms and conditions of MACHEREY-NAGEL, which are printed on the price list. Please contact us if you wish to get an extra copy.

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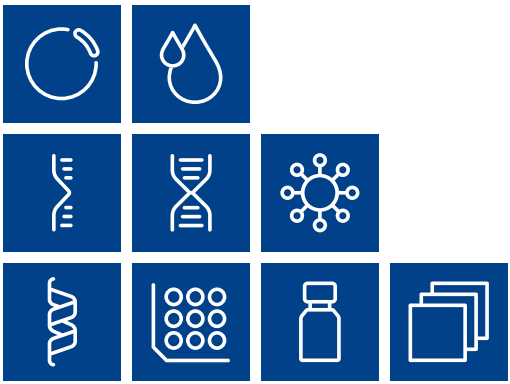
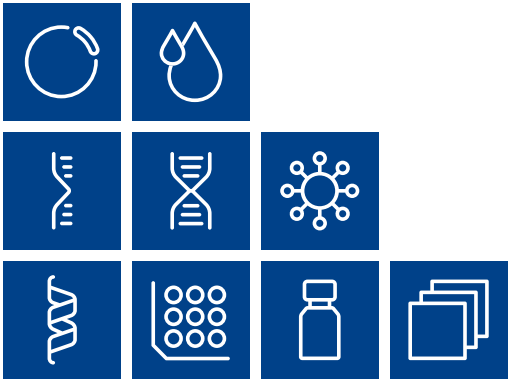
Reason for revision: new document.



Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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