Total RNA and DNA Purification

User Manual

NucleoSpin® RNA/DNA Buffer Set

March 2010 / Rev. 06

MACHEREY-NAGEL

Total RNA/DNA Purification from Tissue/Plant

Protocol-at-a-glance (Rev. 06)

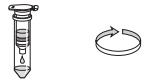
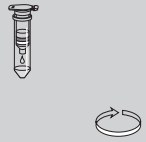
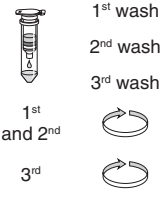
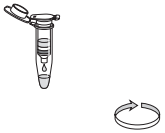
			NucleoSpin® RNA II, NucleoSpin® RNA Plant, NucleoSpin® RNA/Protein	NucleoSpin® RNA XS	
1	**Homogenize sample**		Sample	Sample	
2	**Lyse cells**		350 µl RA1, RAP, or RP1 3.5 µl reducing agent Mix	100 µl RA1 2 µl TCEP Mix	
				5 µl Carrier RNA	
3	**Filtrate lysate**		11,000 x *g* 1 min	11,000 x *g* 30 s	
4	**Adjust RNA binding conditions**		350 µl 70% ethanol Mix	100 µl 70% ethanol Mix	
5	**Bind RNA/DNA**		Load lysate 11,000 x *g* 30 s	Load lysate 11,000 x *g* 30 s	
A	**Wash silica membrane**		1st wash 500 µl *DNA Wash* 2nd wash 500 µl *DNA Wash* 11,000 x *g* 1 min	400 µl *DNA Wash* 400 µl *DNA Wash* 11,000 x *g* 1 min	NucleoSpin® RNA/DNA Buffer Set
B	**Dry membrane**		RT, 3 min	RT, 3 min	
C	**Elute DNA**		100 µl *DNA Elute* 11,000 x *g* 1 min	80 µl *DNA Elute* 11,000 x *g* 1 min	
7	**Digest DNA**		95 µl DNase reaction mixture RT, 15 min	25 µl DNase reaction mixture RT, 15 min	
8	**Wash and dry silica membrane**		1st wash 200 µl RA2 2nd wash 600 µl RA3 3rd wash 250 µl RA3 1st and 2nd 11,000 x *g* 30 s 3rd 11,000 x *g* 2 min	100 µl RA2 400 µl RA3 200 µl RA3 11,000 x *g* 30 s 11,000 x *g* 2 min	
9	**Elute highly pure RNA**		60 µl RNase-free H_2O 11,000 x *g* 1 min	10 µl RNase-free H_2O 11,000 x *g* 30 s	

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Table of contents

1 Components

1.1 Set contents

	NucleoSpin® RNA/DNA Buffer Set
	100 preps
Cat. No.	**740944**
Buffer *DNA Wash* (Concentrate)*	22.5 ml
Buffer *DNA Elute*	12.0 ml
User Manual	1

1.2 Consumables and equipment to be supplied by user

The content of this set is sufficient for 100 DNA isolations in combination with RNA isolations performed with the following kits:

NucleoSpin® RNA II (Cat. No. 740955), NucleoSpin® RNA Plant (Cat. No. 740949), NucleoSpin® RNA/Protein (Cat.No. 740933), NucleoSpin® RNA XS (Cat. No. 740902).

Additional collection tubes are required and are not supplied (see ordering information).

1.3 About this User Manual

It is strongly recommended reading the detailed protocol sections of this User Manual if the **NucleoSpin® RNA/DNA Buffer Set** is used in combination with NucleoSpin® RNA II (Cat. No. 740955), NucleoSpin® RNA Plant (Cat. No. 740949), NucleoSpin® RNA/Protein (Cat.No. 740933), or NucleoSpin® RNA XS (Cat. No. 740902) for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at *www.mn-net.com*.

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

2 Product description

2.1 The basic principle

The **NucleoSpin® RNA/DNA Buffer Set** is intended to be used with one of the following RNA purification kits: NucleoSpin® RNA II, NucleoSpin® RNA Plant, NucleoSpin® RNA/Protein, or NucleoSpin® RNA XS. The combination the **NucleoSpin® RNA/DNA Buffer Set** with either of the RNA purification kits enables the isolation of RNA and DNA from one undivided sample with one single NucleoSpin® RNA Binding Column. This patented technology enables successive elution of DNA and RNA from a NucleoSpin® Column with low salt buffer and water respectively. DNA and RNA are immediately ready for downstream applications. Samples are lysed in the lysis buffer supplied in the NucleoSpin® RNA kits (Lysis Buffer RA1, RAP, or RP1). Ethanol is added to facilitate conditions for binding of nucleic acids to the NucleoSpin® RNA Binding Column. After wash steps DNA and RNA are eluted sequentially. DNA is eluted with a low salt solution (*DNA Elute*) which selectively elutes DNA and keeps RNA on the column. Eluted DNA is immediately ready for downstream applications without further purification. DNA eluted with *DNA Elute* may readily serve as template for PCR, is restrictable with restrictions enzymes and is of high molecular weight (≥ 20 kb). A_{260}/A_{280} ratios of eluted DNA are within a range from 1.7 – 2.0.

After DNA elution, residual on-column-DNA is digested on the NucleoSpin® Column as described in the relating NucleoSpin® RNA protocol. After additional washing steps, pure RNA is eluted with RNase-free water. DNA elution prior to RNA elution does neither compromise RNA quality nor quantity. Sequential DNA and RNA isolation from one sample with this support set and NucleoSpin® RNA kits has been successfully performed with various sample materials (e.g., HeLa cells, pig liver, kidney and spleen, parsley leaf, maize leaf, and root).

The standard protocol (section 5) allows the purification of DNA and RNA from a variety of sample types. Suitable sample types are described in the respective user manuals of the NucleoSpin® RNA kits.

2.2 Kit specifications

Typical yields of total RNA and DNA

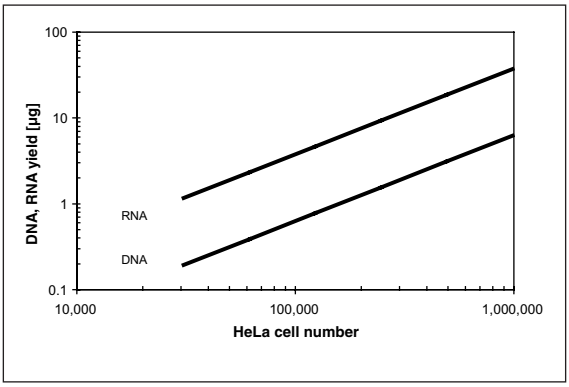

Figure 1: DNA and RNA yield from different amounts of HeLa cells

Different amounts of HeLa cells were used as sample material. DNA and RNA were isolated with the NucleoSpin® RNA/DNA Buffer Set in combination with the NucleoSpin® RNA II kit.

DNA and RNA were isolated as described in Figure 1. Obtained correlation coefficients between sample amount and RNA and DNA yield are shown in Table 1.

Table 1: Correlation between sample amount and nucleic acid yield

	$3 \times 10^4 – 5 \times 10^5$ cells	$3 \times 10^4 – 1 \times 10^6$ cells
RNA	>0.98	>0.98
DNA	>0.99	>0.95

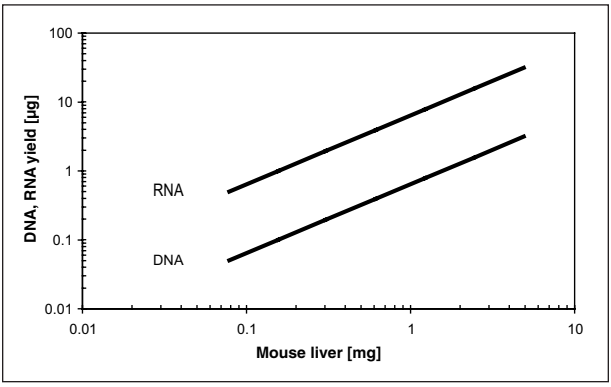

Figure 2: DNA and RNA yield from different amounts of mouse liver tissue

Different amounts of mouse liver tissue were used as sample material. DNA and RNA were isolated with the NucleoSpin® RNA/DNA Buffer Set in combination with the NucleoSpin® RNA II kit.

DNA and RNA were isolated as described in Figure 2. Obtained correlation coefficients between sample amount and RNA and DNA yield are shown in Table 2.

Table 2: Correlation between sample amount and nucleic acid yield

	0.08 – 1.25 mg mouse liver	0.08 – 2.5 mg mouse liver	0.08 – 5 mg mouse liver
RNA	> 0.98	> 0.98	> 0.98
DNA	> 0.99	> 0.95	> 0.67

DNA size and quality

- Isolated genomic DNA is commonly of high molecular weight > 20 kb.
- DNA is commonly stable, even at 37°C for 2 h with or without addition of a typical restriction enzyme buffer.
- DNA is digestable with restriction enzymes.
- DNA is suitable for PCR.

3 Storage conditions and preparation of working solutions

Store solutions at room temperature (18 – 25°C).

- The *DNA Wash* solution is delivered as a concentrate. To prepare the final *DNA Wash* solution, add four volumes of ethanol (50%) to the *DNA Wash* Concentrate (add 90 ml 50% ethanol to 22.5 ml *DNA Wash* Concentrate).
- Due to its composition *DNA Elute* (DNA elution buffer) does not inhibit DNases, i.e. *DNA Elute* does not contain substances (e.g., EDTA) to complex divalent cations. Therefore, make sure not to contaminate *DNA Elute* with DNases!
- Further, due to its composition, *DNA Elute* does not inhibit microbial growth. Therefore, make sure not to contaminate *DNA Elute* with any source of microbial contaminants.

	NucleoSpin® RNA/DNA Buffer Set
	100 preps
Cat. No.	**740944**
Buffer *DNA Wash* (Concentrate)	22.5 ml add 90 ml ethanol (50%)

4 Safety instructions – risk and safety phrases

The **NucleoSpin® RNA/DNA Buffer Set** is intended to be used in conjunction with NucleoSpin® RNA kits. The **NucleoSpin® RNA/DNA Buffer Set** does not contain hazardous contents. However, pay attention to the safety instructions of the individual NucleoSpin® RNA kits!

5 Protocol – Isolation of RNA and DNA from one undivided sample

Before starting the procedure:

- Check if Buffer *DNA Wash* was prepared according to section 3.
- Perform sample homogenization, cell lysis, lysate filtration, adjusting of nucleic acid binding conditions, and binding of nucleic acids to the NucleoSpin® RNA Binding Column according to the NucleoSpin® RNA II, NucleoSpin® RNA Plant, NucleoSpin® RNA/Protein, or NucleoSpin® RNA XS kit standard protocol.

Subsequent to binding of nucleic acids to the column continue as follows with step A (the membrane desalting step of the individual NucleoSpin® RNA protocols is replaced by steps A – C):

A Wash silica membrane

1st wash

+ 500 µl *DNA Wash*

Add **500 µl *DNA Wash*** to the NucleoSpin® RNA Binding Column and centrifuge for **1 min** at **11,000 x *g***. Discard flow-through and reuse Collection Tube.

11,000 x *g* 1 min

If using NucleoSpin® RNA XS add only 400 µl *DNA Wash.*

The *DNA Wash* solution is used instead of MDB (Membrane Desalting Buffer) from the NucleoSpin® RNA kits. MDB will not be used in this procedure.

2nd wash

+ 500 µl *DNA Wash*

Add again **500 µl *DNA Wash*** and centrifuge **1 min** at **11,000 x *g***. Discard Collection Tube with flow-through.

11,000 x *g* 1 min

If using NucleoSpin® RNA XS add only 400 µl *DNA Wash.*

B Dry membrane

Insert the NucleoSpin® RNA Binding Column into a new 1.5 ml microcentrifuge tube (not supplied). Open the lid of the NucleoSpin® RNA Binding Column and let it stand for 3 minutes.

Incubate for 3 min

The procedure ensures complete removal of ethanol from the column.

C Elute DNA

Add **100 µl *DNA Elute*** (DNA elution buffer) directly onto the membrane and incubate 1 min. Elute the DNA by centrifuging for **1 min** at **11,000 x *g***.

Add 100 µl *DNA Elute*

If using NucleoSpin® RNA XS add only 80 µl *DNA Elute* for elution.

The temperature of the DNA Elute solution shall not exceed 30°C, otherwise RNA will partly elute with the DNA Elute *solution.* DNA Elute *solution may stay for 1 min up to 15 min on the column before DNA is eluted. A 1 – 5 min incubation time is recommended. Eluted DNA is immediately ready for downstream applications without further purification.*

11,000 x *g*
1 min

Proceed with the digestion of residual on-column DNA according to the individual NucleoSpin® RNA protocols (step: Digest DNA): Add DNase reaction mixture onto the column and perform all subsequent steps as described in the **NucleoSpin® RNA II, NucleoSpin® RNA Plant, NucleoSpin® RNA/Protein,** or **NucleoSpin® RNA XS** protocol.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
DNA is contaminated with RNA	*Buffer temperature* • DNA elution buffer *DNA Elute* exceeded 30°C during application. Use *DNA Elute* with a temperature preferentially of 18 – 25°C.
DNA yield lower than RNA yield	*Sample material* • DNA and RNA yield depend very much on sample material. Ratio of RNA yield to DNA yield may vary from approximately 1 – 20.
DNA degrades upon storage	*DNase contamination* • DNA elution buffer *DNA Elute* does not contain divalent cations complexing substances (e.g., EDTA). Therefore, DNA is not protected against DNases. Keep *DNA Elute* solution clean and avoid any contamination. As a precaution, keep DNA on ice for short term or at -20°C for long term storage • Some sample materials may contain remaining DNase traces that are not sufficiently washed away by the standard procedure. Perform a wash step of the column with Buffer RA2 after loading the lysate onto the column and before starting the washing steps with *DNA Wash* solution: Add 500 µl Buffer RA2 onto the column, centrifuge 1 min at 11000 x *g* and continue with *DNA Wash* washing steps.
Low RNA yield or quality	*See general protocol* • See troubleshooting section of individual NucleoSpin® protocols. Check if Wash Buffer RA3 has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by Wash Buffer RA3.

Suboptimal performance of DNA in downstream applications	*Divalent cations* • Eluted DNA contains small amounts of divalent cations. If the downstream application comprises for example 50% DNA eluate of the final reaction volume the divalent cations introduced into the reaction by the DNA eluate may alter the performance. Decrease the divalent cation concentration of the reaction by 1 – 5 mM for compensation.
Low DNA yield for large sample amounts	*Sample amount too large* • Depending on the type of sample and its DNA content, DNA yield may not increase proportional with increased sample amount. Sample amounts larger than for example 5 mg tissue or 10^6 cultured cells may yield less DNA than smaller sample amounts. Use smaller sample to ensure good correlation between sample amount and DNA yield.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® RNA/DNA Buffer Set*	740944	100 preps
NucleoSpin® RNA II	740955.20/.50/.250	20/50/250 preps
NucleoSpin® RNA Plant	740949.10/.50/.250	10/50/250 preps
NucleoSpin® RNA/Protein	740933.10/.50/.250	10/50/250 preps
NucleoSpin® RNA XS	740902.10/.50/.250	10/50/250 preps
NucleoSpin® TriPrep*	740666.10/.50/.250	10/50/250 preps
Buffer RA1	740961	50 ml
Buffer RA1	740961.500	500 ml
Buffer RP1	740934.50	50 ml
Buffer RP1	740934.500	500 ml
rDNase Set	740963	1 set

* DISTRIBUTION AND USE OF NUCLEOSPIN® RNA/DNA BUFFER SET and NUCLEOSPIN® TRIPREP IN THE USA IS PROHIBITED FOR PATENT REASONS.

Product	Cat. No.	Pack of
NucleoSpin® Filters	740606	50
NucleoSpin® 96 RNA Filter Plate	740711	4 plates
Collection Tubes (2 ml)	740600	1000

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

6.3 Product use restriction / warranty

NucleoSpin® RNA/DNA Buffer Set components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

DISTRIBUTION AND USE OF THE **NUCLEOSPIN® RNA/DNA BUFFER SET** IN THE USA IS PROHIBITED FOR PATENT REASONS.

It is rather the responsibility of the user to verify the use of the **NucleoSpin® RNA/DNA Buffer Set** for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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