Total RNA and DNA Purification

User manual

NucleoSpin® RNA/ DNA buffer set

April 2005/Rev. 02

MACHEREY-NAGEL

Protocol at a glance (Rev. 02)

Total RNA/DNA Purification from Tissue/Plant

Mini

NucleoSpin® RNA II / NucleoSpin® RNA Plant

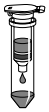
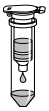
1	Homogenization of sample		30 mg/100 mg	
2	Cell Lysis		350 µl RA1 3.5 µl β-mercaptoethanol or 350 µl RAP 3.5 µl β-mercaptoethanol Mix	
3	Filtration of lysate	 	1 min 11,000 x g	
4	Adjust RNA binding conditions	350 µl 70 % ethanol		
5	Bind RNA/DNA	 	30 sec 8,000 x g	
6	Column wash		1st wash 200 µl *DNA wash* 2nd wash 600 µl *DNA wash* 1 min 11,000 x *g*	NucleoSpin® RNA/DNA buffer set
7	Dry membrane		3 min RT	
8	DNA elution		100 µl *DNA elute* 1 min 11,000 x g	
9	Digest DNA	95 µl DNase reaction mixture RT, 15 min		
10	Wash and Dry silica membrane		1st wash 200 µl RA2 2nd wash 600 µl RA3 3rd wash 250 µl RA3	
		1st and 2nd 	30 sec 8,000 x *g*	
		3rd 	2 min 11,000 x g	
11	Elute highly pure RNA	 	60 µl H_2O (RNase free) 1 min 11,000 x g	

Table of contents

1 Set contents

	NucleoSpin® RNA/ DNA buffer set
	100 preps
Cat. No.	**740944**
Buffer *DNA wash* (concentrate)[1]	22.5 ml
Buffer *DNA elute*	12.0 ml
Protocol	1

The content of this set is sufficient for 100 DNA isolations in combination with NucleoSpin® RNA II (cat.no. 740955) or NucleoSpin® RNA Plant kit (cat.no. 740949). Additional collecting tubes and DNA elution tubes are required and are not supplied.

[1] For preparation of working solutions and storage conditions see section 3.

2 Product description

2.1 The basic principle

The Support Set for RNA and DNA isolation with NucleoSpin® kits is intended to be used in conjunction with the NucleoSpin® RNA II or the NucleoSpin® RNA Plant kit for isolation of RNA and DNA from one undivided sample with one single NucleoSpin column. This patent pending technology enables successive elution of DNA and RNA from a NucleoSpin® column with low salt buffer and water respectively. DNA and RNA are then immediately ready for downstream applications. According to the NucleoSpin® RNA II or NucleoSpin® RNA Plant protocol samples are lysed in lysis buffer RA1 or RAP. 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). $A_{260/280}$ ratios of eluted DNA are within a range from 1.70 – 2.00.

After DNA elution, residual on-column-DNA is digested on the NucleoSpin® column as described in the 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 RNA 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 4.1) allows the clean-up of up to 100 µg of RNA per NucleoSpin® RNA Binding Column or the isolation of total RNA from up to 1 x 10^5 cultured cells (section 4.2).

2.2 About this user manual

Experienced users who are performing the isolation of RNA and DNA using the **NucleoSpin® RNA/DNA buffer set** in combination with NucleoSpin® RNA II (cat.no. 740955) or NucleoSpin® RNA Plant kit (cat.no. 740949) may refer to the Protocol-at-a-glance instead 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. First-time users are strongly advised to read this user manual.

3 Storage conditions an preparation of working solution

Store solutions at room temperature (20-25°C).

- The *DNA wash* solution is delivered as a concentrate. To prepare the final *DNA wash* solution add four volumes of ethanol (~98%) to the *DNA wash* concentrate (add 90 ml ethanol to 22.5 ml *DNA wash* concentrate).

- Due to its composition the *DNA elute* solution (DNA elution buffer) does not inhibit DNases, i.e. *DNA elute* does not contain substances (e.g. EDTA) to complex divalent cations. Therefore, be aware not to contaminate *DNA elute* with DNases!

	NucleoSpin® RNA/ DNA buffer set
Cat. No.	**100 preps** **740944**
Buffer *DNA wash* (concentrate)	22.5 ml add 90 ml ethanol (~98%)

4 Safety Instructions – risk and safety phrases

The *NucleoSpin® RNA/DNA buffer set* for the isolation with NucleoSpin® RNA Kits does not contain hazardous contents. However, pay attention to the safety instructions of the individual NucleoSpin® RNA kits!

5 Protocols

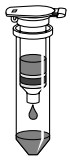
5.1 Isolation of RNA and DNA from one undivided sample

Before starting the procedure, prepare wash buffer *DNA wash* 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** or **NucleoSpin® RNA Plant** kit standard protocol, steps 1-5.

Subsequent to binding of nucleic acids onto the column continue as follows:

A Column wash, 1st

Add **500 µl *DNA wash*** to the column and centrifuge for **1 min** at **11,000 x g**. Discard flow-through and reuse collecting tube.



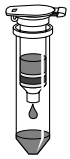
add 500 µl *DNA wash*

The DNA wash solution is used instead of the MDB (membrane desalting buffer) from the NucleoSpin® RNA II or NucleoSpin® RNA Plant kit. MDB will not be used in this procedure here.

1 min
11,000 × *g*

B Column wash, 2nd

Add again **500 µl *DNA wash*** and centrifuge **1 min** at **11,000 x g**. Discard collecting tube with flow-through.



add 500 µl *DNA wash*

1 min
11,000 × *g*

C Dry membrane

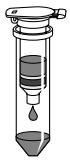
Insert the NucleoSpin® RNA binding column into a fresh 1.5 ml elution tube. **Open the lid** of the spin column and let it stand **for 3 minutes**.

incubate for 3 min

The procedure ensures complete removal of ethanol from the column.

D DNA elution

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**.



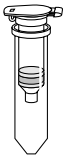
add 100 µl *DNA elute*

1 min
11,000 × *g*

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.

E Digestion of residual on-column DNA, washing and elution of RNA

Prepare and apply DNase reaction mixture according to the NucleoSpin® RNA protocol (step 7 *Digest DNA*). Add DNase reaction mixture onto the column and **proceed** with all subsequent steps **as described in** the **NucleoSpin® RNA II or NucleoSpin® RNA Plant** protocol (steps 8-9).



+ 95 µl DNase reaction mixture

RT
15 min

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 recalcitrant DNase 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 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 application	*Divalent cations* • Eluted DNA contains small amounts of divalent cations. If the downstream application comprises e.g. 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.

6.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® RNA II	**740955.20**	**20**
NucleoSpin® RNA II	**740955.50**	**50**
NucleoSpin® RNA II	**740955.250**	**250**
NucleoSpin® RNA Clean-up	740948.10	10
NucleoSpin® RNA Clean-up	740948.50	50
NucleoSpin® RNA Clean-up	740948.250	250
NucleoSpin® RNA L	740962.20	20
NucleoSpin® RNA Plant	**740949.20**	**20**
NucleoSpin® RNA Plant	**740949.50**	**50**
NucleoSpin® RNA Plant	**740949.250**	**250**
NucleoSpin® 8 RNA	740698	12 x 8
NucleoSpin® 8 RNA	740698.5	60 x 8
NucleoSpin® 96 RNA	740709.2	2 x 96
NucleoSpin® 96 RNA	740709.4	4 x 96
NucleoSpin® 96 RNA	740709.24	24 x 96
Lysis buffer RA1	740961	50 ml
Lysis buffer RA1	740961.500	500 ml
DNase I set	740963	1 set
NucleoSpin® Filter	740606	50
NucleoSpin® collection tubes	740600	1000
NucleoSpin® 96 RNA Filter Plate	740711	4 plates

6.3 Product use restriction / warranty

The **NucleoSpin® RNA/DNA buffer set** components were developed, designed 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).

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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Please contact:

MACHEREY-NAGEL Germany
Tel.: +49-2421/969 270 and 275
e-mail: TECH-BIO@mn-net.com