Parallel isolation of RNA and DNA from unsplitted samples in one working procedure using the NucleoSpin® RNA/DNA buffer set.

← gDNA

← 9,4 kb

DNA eluate

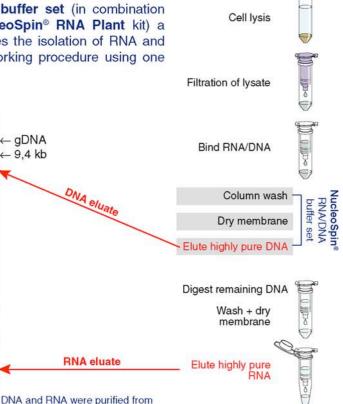
RNA eluate

5 x 106 HeLa cells; 15% of each eluate were analyzed by gel

electrophoresis.

Selective isolation of RNA and DNA from unique, precious samples is a demand for an increasing number of applications. Splitting is unfavourable if not impossible for a number of samples like biopsy samples or single individuals of small organisms (e.g. insects, worms).

With the new NucleoSpin® RNA/DNA buffer set (in combination with the NucleoSpin® RNA II or NucleoSpin® RNA Plant kit) a methodology was developed that enables the isolation of RNA and DNA from unsplitted samples in one working procedure using one silica-based spin column.



Homogenization

of sample

NucleoSpin® RNA/DNA buffer set

- RNA and DNA from one lysate with one column!
 - important for indivisible samples (e.g biopsy material)
 - only one experiment, fast procedure

DNA gel TAE gel

RNA gel

28S rRNA →

18S rRNA →

Denaturing formaldehyd gel

nuclear precursor RNA →

⇒ High quality DNA

- eluted in low salt buffer (<10 mM bivalent cation, pH 7)
- PCR-grade, digestable with restriction enzymes
- 65 80% of yield compared to specialized DNA kit
- A_{260/280} 1,9 to 2,3

High quality RNA

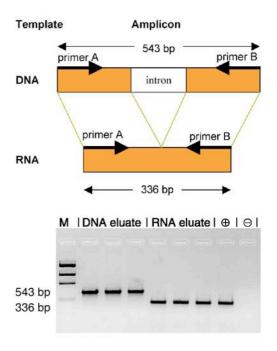
- RNA suitable for all common downstream applications, e.g. RT-PCR, TagMan analysis, blotting, or microarray analysis
- Patent pending
- Cost efficient as it can replace an additional DNA kit



Handling

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. 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). 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.



sample type	sample amount (mg) or (cell number)	DNA yield (µg)	DNA ratio A _{260/280}	RNA yield (µg)	RNA ratio A _{260/280}
HeLa cells	approx. 1x 106	5	2.0	20	2.2
HeLa cells	approx. 7x 10 ⁵	3	2.0	11	2.1
kidney, pig	10 mg	3	2.1	12	2.2
kidney, pig	20 mg	3	2.1	15	2.2
liver, pig	20 mg	7	2.2	51	2.2
liver, pig	30 mg	16	2.2	45	2.2
spleen, pig	20 mg	8	2.1	21	2.0
spleen, pig	30 mg	10	2.0	18	2.1
maize leaf	100 mg	5	2.0	77	2.3
maize root	100 mg	1	1.9	15	2.1
parsley leaf	100 mg	8	2.1	25	2.2

Purified DNA and RNA is well suited for PCR and RT-PCR analysis respectively as shown in Fig.1. An intron spanning primer pair for the human ADP ribosylation factor 1 (ARF1 gene) produces a 336 bp intronless fragment using an aliquot of the RNA elution fraction as template in RT-PCR. The ubiquitously expressed ARF1 gene is of low abundance according to analysis of number of clones obtained from various cell types. No intron containing fragment of 543 bp is generated in the RT-PCR thus indicating very low level of contaminating genomic DNA. Using an aliquot of the DNA elution fraction as template for RT-PCR results in an amplification of a 543 bp intron containing fragment of genomic origin without detectable amplification of the 336 bp intronless fragment. This indicates that DNA is not contaminated with interfering amounts of RNA and that the low salt elution buffer DNA elute does not interfere with PCR.

Ordering Information:

Product	Cat. No.	Distributed by:	0/0/6.2005
NucleoSpin® RNA/DNA buffer set (100 preps)	740 944		1,1dg 1
NucleoSpin® RNA II (20/50/250 preps)	740955.20 / .50 / .250		ADNAbuffe
NucleoSpin® RNA Plant (20/50/250 preps)	740949.20 / .50 / .250		NuSpinBNADNAbuffe

For more information regarding the use of MN products, please contact your local representative or visit MN directly under www.mn-net.com.

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