NucleoSpin® RNA Plant procedure

lysis of the cells

homogenisation by filtration (NucleoSpin® Filter)

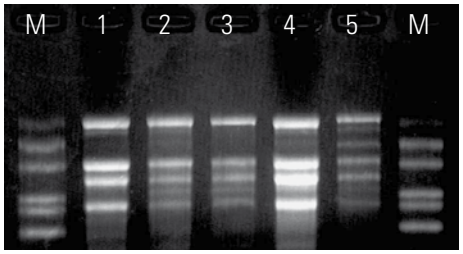
binding

MDB wash

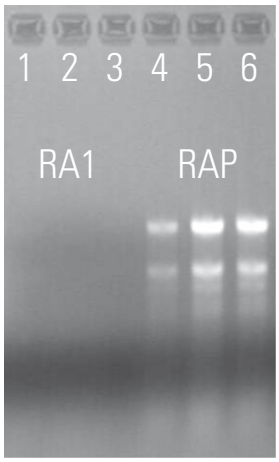
DNase I incubation directly on the membrane at RT

washing

elution



High-quality RNA from plant material
RNA was purified from different plant species (50mg each of leaf samples) using NucleoSpin® RNA Plant. Aliquots of each eluate were loaded onto a 1% formaldehyde agarose gel. Samples: maize (1), thyme (2), Palma Christi (3), tobacco (4), rye (5), M: marker.



Flexibility in lysis system
Due to the very different and difficult compounds of plants an additional lysis buffer may be a solution for higher yields. Macherey-Nagel offers the flexibility of two lysis systems. Total RNA was purified from 50mg seed vessels of poppy seed (Papaver sp.) using the NucleoSpin® RNA Plant kit. With both lysis buffers RA1 (1 to 3) resp. RAP (4 to 6) three preps have been performed in parallel. 20µL of each eluate (elution volume 100µL) was used for RNA quantification via A_{260} and analysed on a 1.2% formaldehyde agarose gel.

Lanes		Average yield
1 to 3:	Poppy seed lysed with buffer RA1	0.84µg
4 to 6:	Poppy seed lysed with buffer RAP	7µg

The use of the alternative lysis buffer RAP gave a much higher yield for poppy seed.

Isolation kits, plant and fungi RNA, NucleoSpin® RNA Plant

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MACHEREY-NAGEL

NucleoSpin® RNA Plant is designed for isolation of DNA-free high quality RNA from a wide variety of plant and fungal samples.

- Silica membrane technology
- Yield: 3µg to 70µg from 100µg plant material
- Sample material: 1mg to 100mg tissue
- Elution volume: 60µL
- Fragment size: 200b to 20kb
- Binding capacity: 200µg
- Preparation time: 30min/6 preps
- Format: mini spin column
- DNase I included
- NucleoSpin filter columns included for homogenisation of lysate and reduction of viscosity
- Parallel purification of DNA possible by using the NucleoSpin RNA/DNA buffer set

Cells are lysed by incubation in a solution containing chaotropic ions. This lysis buffer inactivates RNases creating appropriate binding conditions favouring adsorption of RNA to the silica membrane. After lysis, homogenisation and reduction of viscosity are achieved by filtration with NucleoSpin® filter units. Contaminating DNA bound to the membrane is removed by a DNase I solution directly applied to the membrane during preparation. Optimal conditions for DNase are achieved by washing the membrane with a specific desalting buffer before treatment. Salts, metabolites and macromolecular cellular components are removed by simple washing with two different buffers. Total RNA is finally eluted with RNase-free water. The NucleoSpin® RNA Plant kit features two alternative lysis buffers: in most cases, use of buffer RA1 is recommended for lysis due to its strong denaturing properties. The presence of peculiar metabolites in a variety of plant tissues or fungi may lead to solidification of the lysate, resulting in non-processible slurry. In such cases, the alternative buffer, RAP is the buffer of choice. For thorough homogenisation and reduction of lysate viscosity, NucleoSpin® filter units are also provided.

Kit components: NucleoSpin® RNA columns with 2mL collecting tubes, 2mL collecting tubes, 1.5mL microcentrifuge tubes, NucleoSpin® filters, buffers, RNase-free DNase I, DNase I reaction buffer, RNase-free water.

Average yields of total RNA per 50mg sample (wet weight)

Species	Organ	Yield, µg
Allium cepa	Germ bud	13
Allium sativum	Leaf	13
Arabidopsis thaliana	Leaf	15
Beta vulgaris	Leaf	17
Brassica napus	Leaf	9
	Blossom	9
	Stalk	7
Capiscum annuum	Leaf	8
Cucumis melo	Leaf	15
Gladiolus spec.	Leaf	7
Hordeum vulgare	Leaf	3
Lactuca sativa	Leaf	4
Lycopersicum esculentum	Leaf	10
Mucor rouxii (fungus)	Mycelium	6
Nicotiana tabacum	Leaf	24
	Root tip	12
	Stalk	18
	Blossom	33
Secale cereale	Leaf	12
Taraxacum officinale	Leaf	12
Thymus herba-barona	Leaf	15
Triticum aestivum	Leaf	4
Viola tricolor	Leaf	9
Zea mays	Leaf	18

Catalogue No	Alt. No	Preps per kit
NZ74094950	740949.50	50
NZ740949250	740949.250	250

Accessories

Catalogue No	Alt. No	Description	Quantity, mL	Pack qty
NZ740961	740961	Lysis buffer RA1	50	1
NZ740961500	740961.500	Lysis buffer RA1	500	1
NZ740963	740963	DNase I set, including DNase I and DNase reaction buffer for 50 minipreps of total RNA	-	1
NZ740606	740606	NucleoSpin® filters for filtration of cell and tissue homogenates	-	50
NZ740711	740711	NucleoSpin® RNA filter plate for filtration of cell and tissue homogenates, for use under vacuum or centrifugation	-	4