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1 Protocols

1.1 RNA purification from animal tissue and cultured cells with separation in small and large RNA

Before starting the purification, check that 96–100 % ethanol is available.

Please note that for the separation of small and large RNA, two columns are used for one isolation. The procedure results in 25 isolations for each fraction (50 prep kit). If this procedure is used exclusively, a separate column/buffer set is available to realize 50 isolations for each fraction (see ordering information of NucleoSpin® miRNA user manual).

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1 Cell lysis

See section 3.3 of user manual for more information on homogenization methods.

Animal tissue

Thoroughly grind **animal tissue** under **liquid nitrogen** to a fine powder. Transfer up to **30 mg** to a 1.5 mL microcentrifuge tube (not provided) and add **300 µL Buffer ML**. Pipette up and down (> 5 times) or vortex to lyse the cells.

Disrupt sample

+ 300 µL ML

Alternatively, add **300 µL Buffer ML** to **30 mg animal tissue** and use a **rotor-stator, bead-mill,** or other devices to disrupt the cells.

Incubate for **5 min** at **room temperature (18–25 °C)**.

RT
5 min

Cultured animal cells

Collect up to **10^7 cultured cells** by centrifugation and add **300 µL Buffer ML**. Pipette cells up and down (> 5 times) or vortex to lyse the cells.

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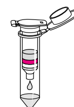
2 Homogenization of the lysate

Place a **NucleoSpin® Filter** (violet ring) into a collection tube (2 mL, lid). Load the mixture and centrifuge for **1 min** at **11,000 x *g*** to reduce viscosity and clear the lysate from undissolved debris.



Load sample

Upon pellet formation in the collection tube (depending on amount/nature of the sample) transfer the supernatant to a new 1.5 mL microcentrifuge tube (not provided) without disturbing the pellet.

11,000 x *g*
1 min

Alternative: Soft samples can be homogenized by passing them > 5 times through a 0.9 mm needle (20 gauge) fitted to a syringe.

3 Adjust binding conditions for large RNA and DNA

Discard the NucleoSpin® Filter. Add **exactly 150 µL 96–100 % ethanol** to **300 µL** homogenized lysate, close the lid, and vortex immediately for **5 s**.

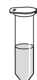
Incubate for **5 min** at **room temperature (18–25 °C)**.

Note: After addition of ethanol a precipitate may become visible. Do not centrifuge the ethanolic lysate and be sure to load all of the precipitate onto the column in step 4.

Note: More than 300 µL of the lysate can be processed, however, the volumes of ethanol (step 3), Buffer MP (step 7) and Buffer MX (step 9) have to be increased proportionally and multiple loading steps might be necessary.

300 µL lysate

+ 150 µL 96–100 % ethanol



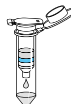
Vortex 5 s

RT
5 min

4 Bind large RNA and DNA

Place a **NucleoSpin® RNA Column** (blue ring) in a Collection Tube (2 mL, lid) and load the sample including any precipitate onto the column.

Centrifuge for **1 min** at **14,000 x *g***.



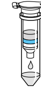
Load sample

Save the flow-through containing the small RNA for step 7. Transfer the NucleoSpin® RNA Column into a new Collection Tube (2 mL) and proceed with **→ step 5** to digest the DNA on the column.

14,000 x *g*
1 min

Note: If you do not want to purify the large RNA fraction, discard the NucleoSpin® RNA Column, save the flow-through containing the small RNA and proceed with **→ step 7**.

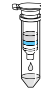
5 Desalt silica membrane



Add **350 µL Buffer MDB** to the NucleoSpin® RNA Column (blue ring) and centrifuge for **1 min** at **11,000 x *g***.

Discard the flow-through and place the column back into the collection tube.

6 Digest DNA

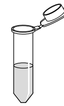


Add **100 µL rDNase** directly onto the silica membrane of the NucleoSpin® RNA Column (blue ring). Do not close the lid.

Incubate at **room temperature (18–25 °C)** until steps 7–10 are completed but at least **15 min**.

7 Precipitate protein

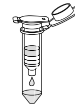
Note: The isolated protein can be easily dissolved in Laemmli buffer and used for SDS-PAGE, Western Blots, and protein quantification. See section 3.4 for detailed information.



Add **300 µL Buffer MP** to the saved flow-through of step 4 containing only protein and small RNA, close the lid, and vortex for **5 s**.

Centrifuge for **3 min** at **11,000 x *g*** to pellet protein. Use the protein pellet for further analysis of the protein fraction.

8 Collect/remove protein precipitate



Place a **NucleoSpin® Protein Removal Column** (white ring) in a Collection Tube (2 mL, lid) and pipette or pour the supernatant containing small RNA and residual protein precipitate onto the column.

Centrifuge for **1 min** at **11,000 x *g*** to remove the residual protein precipitate. Discard the NucleoSpin® Protein Removal Column and **keep the flow-through.**

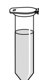
Alternative: If the protein is not to be used, the entire sample including any precipitate can be pipetted or poured onto the NucleoSpin® Protein Removal Column and separated by centrifuging for 3 min at 11,000 x g.

9 Adjust binding conditions for small RNA

Add **800 µL Buffer MX**, close the lid, and vortex for **5 s**.

Note: After addition of Buffer MX a precipitate may become visible. Do NOT centrifuge the mixture and be sure to load all of the precipitate onto the column in step 10.

Note: The yield of small RNA from < 10^6 cells or < 3 mg tissue can be increased by addition of 10 µg Carrier RNA (see section 3.2 for detailed information).



+ 800 µL MX

Vortex 5 s

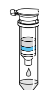
10 Bind small RNA

Place a new **NucleoSpin® RNA Column** (blue ring) in a Collection Tube (2 mL) and load **725 µL** sample onto the column. Centrifuge for **30 s** at **11,000 x *g***.

Discard the flow-through and place the column back into the collection tube.

Repeat this step to **load the remaining** sample.

Load 725 µL sample



11,000 x *g* 30 s

Load remaining sample

11,000 x *g* 30 s

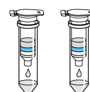
11 Wash and dry silica membrane

1st wash

Add **600 µL Buffer MW1** to each NucleoSpin® RNA Column.

Centrifuge for **30 s** at **11,000 x *g***.

Discard flow-through and place the column back into the collection tube.



+ 600 µL MW1

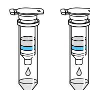
11,000 x *g* 30 s

2nd wash

Add **700 µL Buffer MW2** to each NucleoSpin® RNA Column.

Centrifuge for **30 s** at **11,000 x *g***.

Discard flow-through and place the column back into the collection tube.



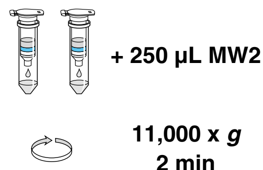
+ 700 µL MW2

11,000 x *g* 30 s

3rd wash

Add **250 µL Buffer MW2** to each NucleoSpin® RNA Column.

Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane completely.

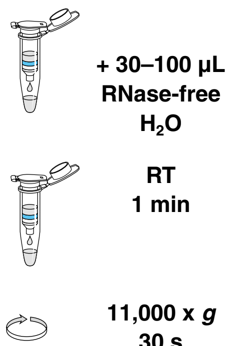


If the liquid in the collection tube has touched the NucleoSpin® RNA Columns after the 3rd wash, discard flow-through and centrifuge again.

Note: The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If new collection tubes are to be used for each step, see ordering information.

12 Elute RNA

Place each NucleoSpin® RNA Column in a new Collection Tube (1.5 mL).



Note: Yield and concentration of isolated RNA are highly dependent on the elution volume. See section 3.5 for detailed information and alternative elution procedures.

Add **30 µL** (for high concentration), **50 µL** (for medium concentration and yield) or **100 µL** (for high yield) **RNase-free H_2O** to the column.

Do not close the lid. Incubate for **1 min** at **room temperature (18–25 °C)**.

Close the lid and centrifuge for **30 s** at **11,000 x *g***.

1.2 RNA purification from plant tissue

Before starting the purification, check that 96–100 % ethanol is available.

Please note that for the separation of small and large RNA, two columns are used for one isolation. The procedure results in 25 isolations for each fraction (50 prep kit).

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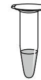
1 Cell lysis

See section 3.3 for more information on homogenization methods.

Disrupt sample

Thoroughly grind **plant tissue** under **liquid nitrogen** to a fine powder. Transfer up to **50 mg** to a 1.5 mL microcentrifuge tube (not provided) and add **400 µL Buffer ML**. Pipette up and down (>5 times) or vortex to lyse the cells.



+ 400 µL ML

Alternatively, add **400 µL Buffer ML** to **50 mg plant tissue** and use a **rotor-stator, bead-mill**, or other devices to disrupt the cells.

Incubate for **5 min** at **room temperature (18–25 °C)**.

RT
5 min

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2 *Optional: Phenol:chloroform extraction*

<u>Note</u>: The organic extraction might help to improve the lysis efficiency and yield for difficult sample material.

Add **1 volume** of **acidic (pH 4.5–4.7) phenol : chloroform** to the sample.

Vortex thoroughly for **30 s**.

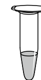
Centrifuge for **10 min** at **11,000 x *g*** to separate the phases.

Transfer the upper aqueous phase without any traces of phenol:chloroform to a new 1.5 mL microcentrifuge tube (not provided).

Proceed directly with **→ step 4**.

Optional:

+ 1 vol acidic phenol: chloroform



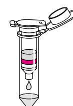
Vortex 30 s

11,000 x *g*
10 min

Separate phases

3 Clarification of the lysate

Place a **NucleoSpin® Filter** (violet ring) in a Collection Tube (2 mL, lid). Load the mixture and centrifuge for **1 min** at **11,000 x *g*** to clear the lysate from undissolved debris.



Load sample

Upon pellet formation in the collection tube (depending on amount/nature of the sample) transfer the supernatant to a new 1.5 mL microcentrifuge tube (not provided) without disturbing the pellet.

11,000 x *g*
1 min

4 Adjust binding conditions for large RNA and DNA

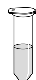
Discard the NucleoSpin® Filter. Add **exactly 150 µL 96–100 % ethanol** to **400 µL flow-through**, close the lid, and vortex immediately for **5 s**.

Incubate for **5 min** at **room temperature (18–25 °C)**.

<u>Note</u>: After addition of ethanol a white or green precipitate may become visible. Do not centrifuge the ethanolic lysate and be sure to load all of the precipitate onto the column in step 5.

<u>Note</u>: More than 400 µL of the lysate can be processed, however, the volumes of ethanol (step 4) and Buffer MX (step 8) have to be increased proportionally and multiple loading steps might be necessary.

400 µL flow-through



+ 150 µL 96–100 % ethanol

Vortex 5 s

RT
5 min

Continue with step 4 of the NucleoSpin® miRNA standard protocol (section 5.1 of user manual) for total RNA isolation and step 4 of the NucleoSpin® miRNA protocol for separation of small and large RNA (page 3).

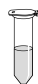
1.3 Fractionation of pre-purified RNA in small RNA and large RNA

Before starting with the preparation check that 96–100% ethanol is available.

1 Prepare sample

Add **150 µL Buffer ML** to **150 µL pre-purified RNA** and vortex for **5 s**.

Note: To purify less than 150 µL, adjust volume with RNase-free water to 150 µL. To process more than 150 µL, increase Buffer ML (step 1), MP (step 4), and MX (step 4) proportionally.



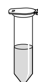
+ 150 µL RNA solution

Vortex 5 s

2 Adjust binding conditions for large RNA

Add **exactly 100 µL 96–100 % ethanol**, close the lid, and vortex for **5 s**.

Incubate for **5 min** at **room temperature (18–25 °C)**.



+ 100 µL 96–100 % ethanol

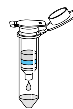
Vortex 5 s

RT 5 min

3 Bind large dsRNA

Place a **NucleoSpin® RNA Column** (blue ring) in a Collection Tube (2 mL, lid) and load the sample onto the column. Centrifuge for **30 s** at **11,000 x *g***.

Transfer the NucleoSpin® RNA Column containing the **large RNA** to a new Collection Tube (2 mL) and save it for step 6.



Load sample

11,000 x *g* 30 s

4 Adjust binding conditions for small RNA

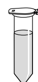
Add **100 µL Buffer MP** to the flow-through of step 3, close the lid, and vortex for **5 s**.

Incubate for **5 min** at **room temperature (18–25 °C)**.

Add **800 µL Buffer MX** and vortex for **5 s**.

+ 100 µL MP

Vortex 5 s



RT 5 min

+ 800 µL MX

Vortex 5 s

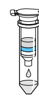
5 Bind small RNA

Place a new **NucleoSpin® RNA Column** (blue ring) in a Collection Tube (2 mL) and load **700 µL** sample onto the column.

Centrifuge for **30 s** at **11,000 x *g***.

Discard the flow-through and place the column back into the collection tube.

Repeat this step to **load the remaining** sample.



Load 700 µL sample

11,000 x *g* 30 s

Load remaining sample

11,000 x *g* 30 s

6 Wash and dry silica membrane

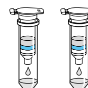
From this step on the **NucleoSpin® RNA Column** containing **large dsRNA** and the **NucleoSpin® RNA Column** containing **siRNA** can be processed simultaneously in the same way.

1st wash

Add **700 µL Buffer MW2** to each NucleoSpin® RNA Column.

Centrifuge for **30 s** at **11,000 x *g***.

Discard flow-through and place the column back into the collection tube.



+ 700 µL MW2

11,000 x *g* 30 s

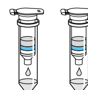
2nd wash

Add **250 µL Buffer MW2** to each NucleoSpin® RNA Column.

Centrifuge for **2 min** at **11,000 x *g*** to dry the membrane completely.

If the liquid in the collection tube has touched the NucleoSpin® RNA/miRNA Column after the 2nd wash, discard flow-through and centrifuge again.

<u>Note</u>: The same collection tube is used throughout the entire washing procedure to reduce plastic waste. If new collection tubes are to be used for each step, see ordering information.

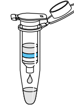


+ 250 µL MW2

11,000 x *g* 2 min

Elute small RNA

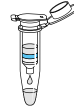
Place each NucleoSpin® RNA Column in a new Collection Tube (1.5 mL).



+ 30–100 µL RNase-free H_2O

Note: Yield and concentration of isolated RNA are highly dependent on the elution volume. See section 3.5 for detailed information and alternative elution procedures.

Add **30 µL** (for high concentration), **50 µL** (for medium concentration and yield) or **100 µL** (for high yield) **RNase-free H_2O** to the column.



RT
1 min

Do not close the lid. Incubate for **1 min** at **room temperature (18–25 °C)**.

11,000 x *g*
30 s

Close the lid and centrifuge for **30 s** at **11,000 x *g***.