

Instructions for Use

Life Science Kits & Assays

innuPREP DNA/RNA Mini Kit

Order No.:

845-KS-20800010 10 reactions

845-KS-20800050 50 reactions

845-KS-20800250 250 reactions

Publication No.: HB_KS-2080_e_170719

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It needs not necessarily agree with future versions. Subject to change!

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1. Introduction

1.1 Intended use

The innuPREP DNA/RNA Mini Kit has been designed for the extraction of DNA and RNA from eukaryotic cells, tissue samples and bacteria. The kit uses an optimized chemistry resulting in a fast and reliable purification of RNA with high quality and yield.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
	REF Catalogue number
	Content Contains sufficient reagents for <N> reactions
	Storage conditions Store at room temperature or shown conditions respectively
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
	Lot number The number of the kit charge
	Manufactured by Contact information of manufacturer
	For single use only Do not use components for a second time
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 3).
- Working steps are numbered.

2. Safety precautions

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. This kit could be used with potential infectious samples. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

3. Storage conditions

The innuPREP DNA/RNA Mini Kit should be stored dry, at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box.

If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature.

For further information see chapter "Kit components" (→ p. 8).

4. Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. This product has been produced and tested in an ISO 13485 certified facility.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP DNA/RNA Mini Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5. Product use and warranty

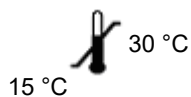
The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ "Intended use" p. 2), (→ "Product specifications" p. 8). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application described above, the user is responsible for the validation of the performance of Analytik Jena AG kits using other protocols than those described below. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE




For research use only!




6. Kit components



IMPORTANT

All kit components are stored at room temperature (15 °C to 30 °C).

	 10	 50	 250
Lysis Solution RL	6 ml	30 ml	125 ml
Washing Solution HS (conc.)	6 ml	30 ml	2 x 70 ml
Washing Solution LS (conc.)	3 ml	2 x 8 ml	2 x 40 ml
RNase-free Water	2 ml	6 ml	25 ml
Elution Buffer	2 ml	6 ml	30 ml
Spin Filter D	10	50	5 x 50
Spin Filter R	10	50	5 x 50
Receiver Tubes	40	4 x 50	20 x 50
Elution Tubes	20	2 x 50	10 x 50
Manual	1	1	1

	 10	 50	 250
Initial steps	Washing Solution HS Add 6 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!	Washing Solution HS Add 30 ml of 96-99.8 % ethanol to the bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!	Washing Solution HS Add 70 ml of 96-99.8 % ethanol to each bottle Washing Solution HS, mix thoroughly and keep the bottle always firmly closed!
	Washing Solution LS Add 12 ml of 96-99.8 % ethanol to the bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!	Washing Solution LS Add 32 ml of 96-99.8 % ethanol to each bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!	Washing Solution LS Add 160 ml of 96-99.8 % ethanol to each bottle Washing Solution LS, mix thoroughly and keep the bottle always firmly closed!

Components not included in the kit

- DNase I; optional
- Lysozyme; optional
- ddH₂O
- TE-Buffer (10 mM Tris-HCl; 1 mM EDTA; pH 8.0); optional
- Reaction tubes
- Ethanol (70 %, 96-99.8 %)

7. Product specifications



1. Starting material:
 - Eukaryotic cells (max. 5×10^6)
 - Tissue sample (up to 20 mg)
 - Bacterial cells (gram+ and gram- bacteria, up to 1×10^9)

2. Time for isolation:
Approximately 15-40 min

3. Typical yield:
 - Depending on the kind and initial amount of the starting material
 - Up to 60 μg RNA and up to 40 μg DNA

4. Binding capacity:
 - Approximately: 100 μg RNA
 - > 50 μg DNA

8. GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases	EUH
Lysis Solution RL	Guanidinium thiocyanate 25–50 %	 <i>Danger</i>	302, 314, 412	101, 102, 103, 260, 303+361+353, 305+351+338, 310, 405, 501	
Washing Solution HS (conc.)	Guanidinium thiocyanate 50–100 %	 <i>Danger</i>	302, 314, 412	101, 102, 103, 260, 310, 405, 501, 303+361+353, 305+351+338	032

CAUTION

DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

8.1 Hazard phrases

- 302 Harmful if swallowed.
- 314 Causes severe skin burns and eye damage.
- 412 Harmful to aquatic life with long lasting effects.

8.2 Precaution phrases

- 101 If medical advice is needed, have product container or label at hand.
- 102 Keep out of reach of children.
- 103 Read label before use.
- 260 Do not breathe dust/fume/gas/mist/vapors/spray.
- 310 Immediately call a POISON CENTER/doctor.
- 405 Store locked up.
- 501 Dispose of contents/container in accordance with local/regional/national/international regulations.
- 303+361+353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
- 305+351+338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

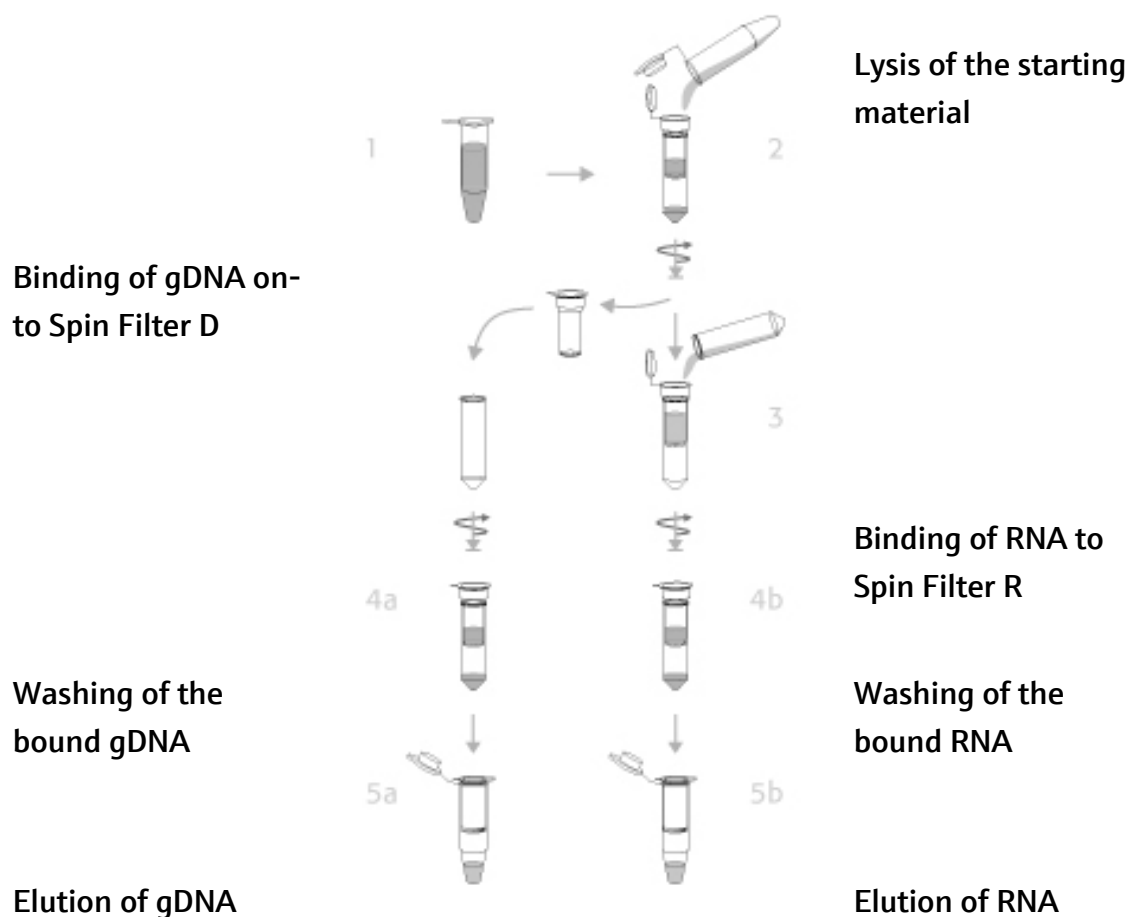
8.3 EU hazard statements

- 032 Contact with acids liberates very toxic gas

9. Recommended steps before starting

- Ensure that the **Washing Solution HS** and **Washing Solution LS** have been prepared according to the instruction (→ "Kit components" p. 6)
- Centrifugation steps should be performed at room temperature
- Avoid freezing and thawing of starting materials

10. General procedure for nucleic acid extraction



11. General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plastic ware should be treated before use to ensure that it is RNase-free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Autoclaving alone will not inactivate many RNases completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0.1 % DEPC solution for 12 hours at 37 °C and then it has to be autoclaved or heated to 100 °C for 15 min to remove residual DEPC.

- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.
- All buffers have to be prepared with DEPC-treated RNase-free ddH₂O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plastic ware employed for other applications which might introduce RNase contaminations in the RNA isolation.

12. Protocol 1: DNA and RNA extraction from tissue samples (up to 20 mg)

IMPORTANT NOTE

Please note that up to 20 mg of tissue samples can be processed.
Avoid freezing and thawing of tissue samples!

I. Homogenization of starting material

NOTE

To maximize the final yield of DNA and total RNA a complete homogenization of tissue sample is important!

For the homogenization of tissue sample it is possible to use commercially available rotor-stator homogenizer or bead mills. It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the tissue sample to a fine powder.

A. Homogenization of the tissue sample using a rotor-stator homogenizer

1. Transfer the weighed amount of fresh or frozen starting material in a suitable reaction vessel for the homogenizer.
2. Add 450 μ l Lysis Solution RL.
3. Homogenize the sample.
4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and place the sample under Lysis Solution RL for longer storage at -20°C or use the sample immediately for isolation of DNA/RNA following the protocol step II.

B. Disruption of the tissue sample using a mortar and pestle and liquid nitrogen

1. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine tissue powder.
2. Transfer the powder into a 1.5 ml reaction tube. Don't allow the sample to thaw!
3. Add 450 μ l Lysis Solution RL and incubate the sample for appropriate time for a further lysis under continuous shaking.
4. Finally place the sample under Lysis Solution RL for longer storage at -20°C or use the sample immediately for isolation of DNA/RNA following protocol step II.

II. Binding of genomic DNA onto Spin Filter D

1. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at $10,000 \times g$ ($\sim 12,000$ rpm) for 2 minutes.

Do not discard the filtrate, because the filtrate contains the RNA!

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

2. Place the Spin Filter D into a new Receiver Tube. The genomic DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R.

III. Binding of total RNA onto Spin Filter R

1. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (appr. 400 μ l) of **70 % ethanol** to the filtrate from step II. Mix the sample by pipetting up and down several times. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with filtrate.

2. Place the Spin Filter R into a new Receiver Tube. The total RNA is bound on Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

IV. Parallel processing of both - Spin Filter D for isolation of DNA and Spin Filter R for isolation of RNA

1. Open the Spin Filters D and R, add **500 μ l Washing Solution HS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
2. Open the Spin Filters D and R, add **700 μ l Washing Solution LS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes. Place the Spin Filters D and R again into the Receiver Tubes.
3. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.

4. Place the Spin Filters D and R each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30–80 µl RNase-free Water** to Spin Filter R. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of genomic DNA or total RNA you can also elute with different volumes of Elution Buffer/RNase-free water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free water should be 20 µl.

Store nucleic acids at appropriate conditions (RNA at –80 °C and DNA at –20 °C)!

13. Protocol 2: DNA and RNA extraction from eucaryotic cells (5×10^6 cells)

IMPORTANT

Please note that up to 5×10^6 cells can be processed.

I. Lysis of cells

Add **400 μ l Lysis Solution RL** to the cell pellet. Incubate for 2 minutes at room temperature. Re-suspend the cell pellet completely by pipetting up and down. Incubate the sample for further 3 minutes at room temperature.

NOTE

To maximize the final yield of DNA and total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

II. Binding of genomic DNA onto Spin Filter D

1. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at $10,000 \times g$ ($\sim 12,000$ rpm) for 2 minutes.

Do not discard the filtrate, because the filtrate contains the RNA!

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

2. Place the Spin Filter D into a new Receiver Tube. The genomic DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R.

III. Binding of total RNA onto Spin Filter R

1. Place a Spin Filter R into a new Receiver Tube. Add an equal volume (appr. 400 µl) of 70 % ethanol to the filtrate from step II. Mix the sample by pipetting sometimes up and down. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

Discard the Receiver Tube with filtrate.

2. Place the Spin Filter R into a new Receiver Tube. The total RNA is bound on Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

IV. Parallel processing of both - Spin Filter D for isolation of DNA and Spin Filter R for isolation of RNA

1. Open the Spin Filters D and R, add 500 µl Washing Solution HS to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
2. Open the Spin Filters D and R, add 700 µl Washing Solution LS to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
3. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.

4. Place the Spin Filters D and R each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30–80 µl RNase-free Water** to Spin Filter R. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of genomic DNA or total RNA you can also elute with different volumes of Elution Buffer/RNase-free Water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free water should be 20 µl.

Store nucleic acids at appropriate conditions (RNA at –80 °C and DNA at –20 °C)!

14. Protocol 3: DNA and RNA extraction from bacterial cells

I. Lysis of bacterial cells

IMPORTANT

Please note that up to 1×10^9 cells can be processed.

We recommend a pre-incubation of bacterial cells with Lysozyme or optionally other bacterial lysis proteins.

Stock solution of Lysozyme for Gram(-) bacteria:

20 mg/ml in water; storage of Lysozyme stock solution in aliquots at -22 °C to -18 °C

Stock solution of Lysozyme for Gram(+) bacteria:

50 mg/ml in water; storage of Lysozyme stock solution in aliquots -22 °C to -18 °C

Prepare TE-Buffer: (10 mM Tris HCl / 1 mM EDTA; pH 8.0)

1. Spin down the bacterial cells by centrifugation at 5,000 x g for 2-5 minutes. Discard the supernatant as complete as possible.
2. For Gram(-) bacteria re-suspend the cell pellet in **100 µl TE-Buffer** **and add 2 µl** of the corresponding **Lysozyme** stock solution. Pipette sometimes up and down; the solution should become clear or viscous.
3. For Gram(+) bacteria re-suspend the cell pellet in **100 µl TE-Buffer** **and add 6 µl** of the corresponding **Lysozyme** stock solution. Pipette sometimes up and down; incubate until the solution becomes clear or viscous.

NOTE

The amount of Lysozyme and also the essential time for incubation may need to be diversified depending on bacterial strains. Read also the guideline of the Lysozyme supplier. A complete destruction of bacterial cell walls is important.

3. Add 450 μ l Lysis Solution RL to the sample and vortex vigorously or pipette sometimes up and down. Incubate the sample for further 3 minutes at room temperature.
-

NOTE

To maximize the final yield of DNA and total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

II. Binding of genomic DNA onto Spin Filter D

1. After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spin Filter D into a Receiver Tube. Transfer the supernatant of the lysed sample onto the Spin Filter D. Centrifuge at 10,000 \times g (~12,000 rpm) for 2 minutes.

Do not discard the filtrate, because the filtrate contains the RNA!

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

2. Place the Spin Filter D into a new Receiver Tube. The genomic DNA is bound onto Spin Filter D. Processing of the Spin Filter D will be continued after binding of total RNA onto Spin Filter R.

III. Binding of total RNA onto Spin Filter R

1. Place a Spin Filter R into a new Receiver Tube. Add an **equal volume** (appr. 600 μ l) of **70 % ethanol** to the filtrate from previous step II. Mix the sample by pipetting sometimes up and down.
2. Transfer **650 μ l of the sample** onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tube. Place the Spin Filter R back into the Receiver Tube. **Load the residual sample** on the Spin Filter R and centrifuge again at 10,000 x g (~12,000 rpm) for 1 minute.

NOTE

If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

3. Discard the filtrate and re-use the Receiver Tube. The total RNA is bound onto Spin Filter R. Both Spin Filters (Spin Filter D and R) will be processed in parallel now.

VI. Parallel processing of both - Spin Filter D for the isolation of DNA and Spin Filter R for isolation of RNA

1. Open the Spin Filters D and R, add **500 μ l Washing Solution HS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
2. Open the Spin Filters D and R, add **700 μ l Washing Solution LS** to each, close the caps and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the filtrate and re-use the Receiver Tubes.
3. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tubes.

4. Place the Spin Filter D and R each into an Elution Tube. Carefully open the caps of the Spin Filters D and R, add **100 µl Elution Buffer** to Spin Filter D and **30–80 µl RNase-free Water** to Spin Filter R. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (~8,000 rpm) for 1 minute.

NOTE

Depending on the extracted yield or the needed concentration of genomic DNA or total RNA you can also elute with different volumes of Elution Buffer/RNase-free Water. A lower volume of Elution Buffer/RNase-free Water increases the concentration of DNA/RNA and a higher volume of Elution Buffer/RNase-free Water leads to an increased yield but a lower concentration of nucleic acids. Please note, that the minimum of RNase-free Water should be 20 µl.

Store nucleic acids at appropriate conditions (RNA at –80 °C and DNA at –20 °C)!

15. Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no DNA or total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with Elution Buffer and RNase-free water to 5 minutes or repeat elution step once again.
DNA contamination of extracted RNA	
Too much starting material	Reduce amount of starting material.
Incorrect lysis of starting material	Use the recommended techniques for lysis of cell pellet. Perform DNase digest of the eluate containing the total RNA or perform a on column DNase digest step after binding of the RNA on Spin Filter R!
Total RNA degraded	
RNA source inappropriately handled or stored	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contamination of solutions; Receiver Tubes, etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream applications (e.g. RT-PCR)	
Ethanol carryover during elution	Increase time for removing of ethanol.
Salt carryover during elution	Ensure that Washing Solution HS and Washing Solution LS are at room temperature. Check up Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

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