Instructions for Use
Life Science Kits & Assays

innuSPEED Tissue RNA Kit

Order No.:
845-KS-2540010 10 reactions
845-KS-2540050 50 reactions
845-KS-2540250 250 reactions

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

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Manufacturer:
AJ Innuscreen GmbH
Robert-Rössle-Straße 10
13125 Berlin
Made in Germany!

Distribution/Publisher:
Analytik Jena AG
Konrad-Zuse-Straße 1
07745 Jena · Germany

Phone +49 3641 77 9400
Fax +49 3641 77 767776
www.analytik-jena.com
info@analytik-jena.com

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1 Introduction

1.1 Intended use

The innuSPEED Tissue RNA Kit has been designed for simple, reliable and fast isolation of total RNA from up to 20 mg tissue using SpeedMill by Analytik Jena for homogenization of starting material. The isolation procedure is based on a new kind of patented technology (called DC chemistry).

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	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15 °C 30 °C	**Storage conditions** Store at room temperature or shown conditions respectively.
i	**Consult instructions for use** This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
2	**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

NOTE

Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

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.

FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION!

Don’t eat or drink components of the kit!

The kit shall only be handled by educated personnel in a laboratory environment!

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.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE
Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information, please ask for the material safety data sheet (MSDS).

3 Storage conditions

The innuSPEED Tissue RNA 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. 7).

4 Functional 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 innuSPEED Tissue RNA 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.

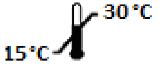
5 Product use and warranty

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. 9). 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



STORAGE CONDITIONS
All components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-2540010	845-KS-2540050	845-KS-2540250
Lysis Tube P	10	50	5 x 50
Lysis Solution RL	6 ml	30 ml	125 ml
Washing Solution HS (conc.)	3 ml	15 ml	70 ml
Washing Solution LS (conc.)	2 ml	8 ml	40 ml
RNase-free Water	2 ml	6 ml	2 x 15 ml
Spin Filter D	10	50	5 x 50
Spin Filter R	10	50	5 x 50
Receiver Tubes	60	6 x 50	30 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

	Σ 10	Σ 50	Σ 250
Initial steps	**Washing Solution HS** Add 3 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed! **Washing Solution LS** Add 8 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution HS** Add 15 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed! **Washing Solution LS** Add 32 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution HS** Add 70 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed! **Washing Solution LS** Add 160 ml of 96-99.8 % ethanol to each bottle and mix thoroughly. Keep the bottle always firmly closed!

Components not included in the kit

- DNase I; optional
- 1.5 ml reaction tubes
- ddH_2O
- Ethanol (70 %, 96–99.8 %); non denatured or methylated
- SpeedMill (homogenizer from Analytik Jena AG) or other type of homogenizer

7 Product specifications

1. Starting material:

   Up to 20 mg fresh or frozen tissue samples.

2. Time for isolation:

   Approximately 15–40 minutes.

3. Typical yield:

   Not determined. The yield depends on the amount and quality of the starting material.

8 GHS classification

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

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 lo-cal/regional/national/international regulations.
303+361+353	IF ON SKIN (or hair): Take off immediately all contam-inated 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.

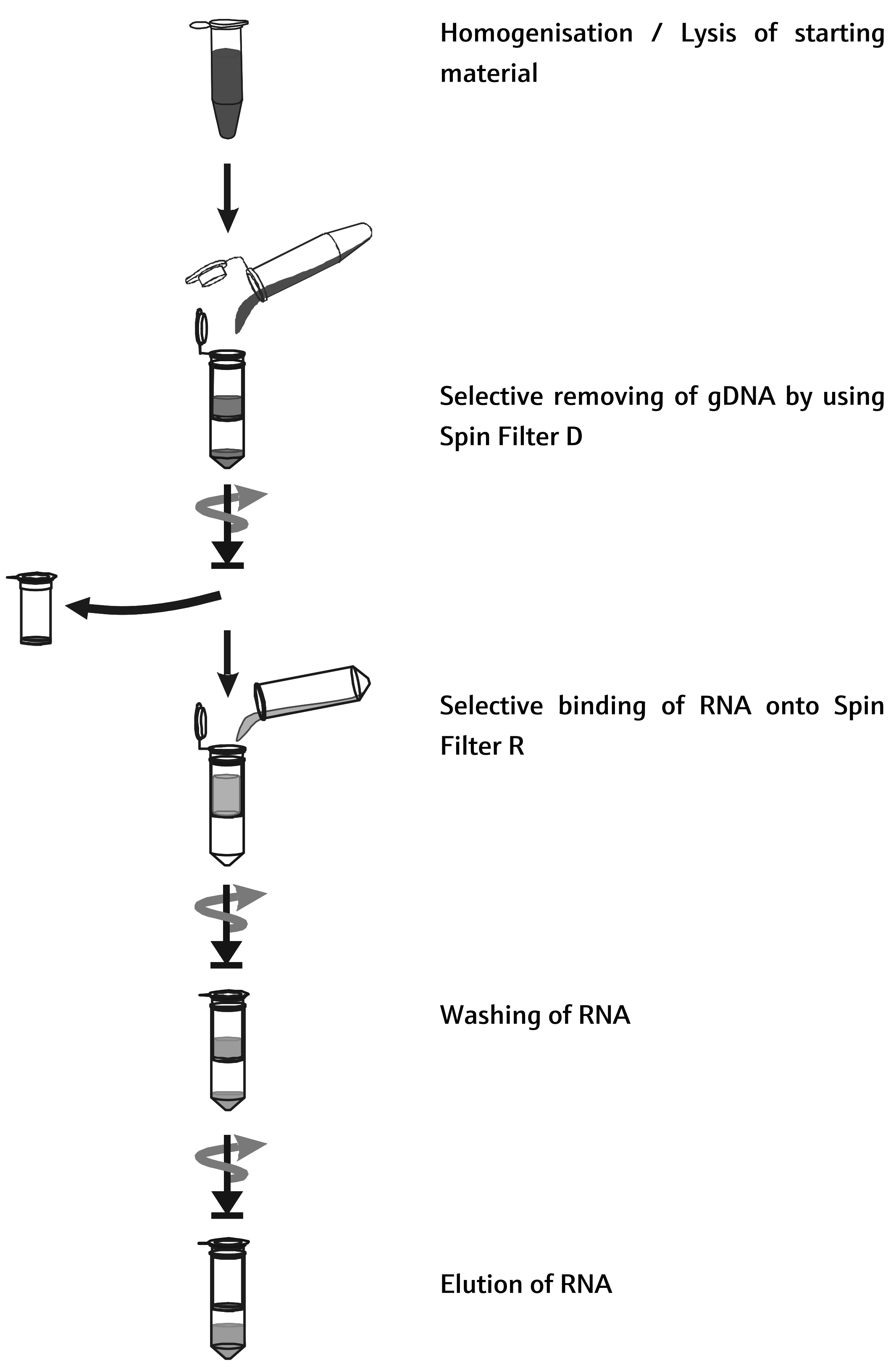
8.3 EU hazard statements

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. 7).
- 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 ddH_2O.
- 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: RNA extraction from tissue samples (up to 20 mg) using SpeedMill

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

A. Homogenization of starting material using SpeedMill

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

1. Cut the starting material into small pieces.

   The following table shows some recommended sizes depending on different groups of tissue material for an optimal homogenization process.

Groups of tissue material	Optimal size for homogenization
Soft tissue material like: lung, kidney, brain, spleen, liver etc.	approx. 5 mm x 5 mm
Very hard material like: rodent tails or cartilage material	approx. 1-1.5 mm

   Table 1: Recommended optimal size of starting material.

   In general, smaller pieces of starting material are better for homogenization.

2. Transfer the cut starting material into the **Lysis Tube P**. Add **100 µl Lysis Solution RL**.

3. Place the **Lysis Tube P** in the SpeedMill and start the homogenization process. Time for processing depends on type of starting material.

   The following table shows a short overview about processing time depending on different types of starting material.

Groups of tissue material	Recommended time for homogenization
Soft tissue material like: lung, kidney, brain, spleen, liver etc.	approx. 0,5–1 minute
Very hard material like: rodent tails or cartilage material	approx. 2 x 2 minutes
Other materials: e.g. insects like ticks	approx. 2 x 2 minutes

Table 2: Recommended time for homogenization.

NOTE
If starting material is not homogenized, please increase the homogenization time.

B. Extraction procedure

1. After homogenization please check, that the starting material is completely disrupted. Open the Lysis Tube P and add **350 µl** of **Lysis Solution RL**. Incubate at room temperature for 15 minutes.

   Continuous shaking during the incubation time will increase the extraction efficiency.

2. 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 x g (~12.000 rpm) for 2 minutes. Discard the Spin Filter D.

   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.

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3. 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 2. Mix the sample by pipetting several times up and down. Transfer the sample onto the Spin Filter R. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes.

   Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.

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

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4. Open the Spin Filter R and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
5. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
6. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
7. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **30–80 µl RNase-free Water**. 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 total RNA you can also elute with different volumes of RNase-free Water. A lower volume of RNase-free water increases the concentration of RNA and a higher volume of RNase-free Water leads to an increased yield but a lower concentration of total RNA. Please note, that the minimum of RNase-free water should be 20 µl.

13 Protocol 2: RNA extraction from tissue material (up to 20 mg) using other homogenizers

IMPORTANT

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

For the homogenization of tissue samples it is possible to use commercially available homogenizer which work with 2.0 ml "Grinding Tubes" e.g. the homogenizer "Precellys" or the homogenizer "FastPrep"!

A. Homogenization of starting material using other homogenizers

1. Cut the starting material into small pieces.

   Efficiency of homogenization process depends on the size of starting material.

2. Transfer the cut starting material into the **Lysis Tube P**. Add **100 µl Lysis Solution RL** and place the **Lysis Tube P** in a homogenizer and start the homogenization process according to the instruction manual of the homogenizer.

NOTE

The time for homogenization and the power of homogenization depends on the kind of homogenizer used. Normally 20 seconds to 1 minute are sufficient for complete disruption of tissue material. Furthermore, the efficiency of homogenization depends on kind of tissue material. Please find the individual parameter for the specific application!

B. Extraction procedure

1. After homogenization please check, that the starting material is completely disrupted. Open the Lysis Tube P and add **350 µl** of **Lysis Solution RL**. Incubate at room temperature for 15 minutes.

   Continuous shaking during the incubation time will increase the extraction efficiency. Alternatively, vortex the tube two times for 5 seconds.

2. 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 x g (~12,000 rpm) for 2 minutes. Discard the Spin Filter D.

   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.

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

   Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.

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

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4. Open the Spin Filter R and add **500 µl Washing Solution HS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
5. Open the Spin Filter R and add **700 µl Washing Solution LS**, close the cap and centrifuge at 10,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
6. Centrifuge at 10,000 x g (~12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
7. Place the Spin Filter R into an Elution Tube. Carefully open the cap of the Spin Filter R and add **30–80 µl RNase-free Water**. 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 total RNA you can also elute with different volumes of RNase-free Water. A lower volume of RNase-free Water increases the concentration of RNA and a higher volume of RNase-free Water leads to an increased yield but a lower concentration of total RNA. Please note, that the minimum of RNase-free Water should be 20 µl.

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14 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 total RNA eluted	
Insufficient disruption or homogenization	Reduce amount of starting material. Overloading reduces yield!
Incomplete elution	Prolong the incubation time with RNase-free Water to 5 minutes or repeat elution step once again.
DNA contamination	
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 an 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. Checkup Washing Solution for salt precipitates. If there are any precipitate dissolves these precipitate by carefully warming.

15 Related products

Name	Amount	Order No.
Products for PCR & Gel Electrophoresis		
innuPREP DOUBLEpure Kit	10 rxn	845-KS-5050010
	50 rxn	845-KS-5050050
	250 rxn	845-KS-5050250
innuPREP Gel Extraction Kit	10 rxn	845-KS-5030010
	50 rxn	845-KS-5030050
	250 rxn	845-KS-5030250
innuPREP PCRpure Kit	10 rxn	845-KS-5010010
	50 rxn	845-KS-5010050
	250 rxn	845-KS-5010250
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
50x inNucleotide Mix (1.5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006

Headquarters

Analytik Jena AG
Konrad-Zuse-Str. 1
07745 Jena · Germany

Phone +49 3641 77 70
Fax +49 3641 77 9279
info@analytik-jena.com
www.analytik-jena.com

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Subject to changes in design and scope of delivery as well as further technical development.

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