Instructions for Use
Life Science Kits & Assays

innuPREP Plant RNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-2060010 10 reactions
845-KS-2060050 50 reactions
845-KS-2060250 250 reactions

Publication No.: HB_KS-2060_e_170713

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AJ Innuscreen GmbH
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Contents

1. Introduction

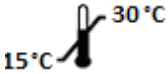
1.1 Intended use

The innuPREP Plant RNA Kit has been designed for simple, reliable and fast isolation of total RNA from different kinds of plant material. The extraction procedure is based on a new kind of patented technology (called DC chemistry). The innuPREP Plant RNA Kit is optimized for the rapid preparation of highly pure RNA from plant material.

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

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

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 personal 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. Analytik Jena AG has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions. 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 innuPREP Plant 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 innuPREP Plant 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

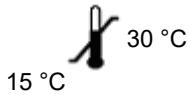
The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use“ p. 2), (→ “Product specifications“ p. 9). Since the performance characteristics of Analytik Jena AG kits have just been validated for the application 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 the 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



STORGAGE CONDITIONS

All components are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-2060010	845-KS-2060050	845-KS-2060250
Lysis Solution RL	6 ml	30 ml	125 ml
Lysis Solution PL	6 ml	30 ml	125 ml
Washing Solution HS (conc.)	3 ml	15 ml	70 ml
Washing Solution LS (conc.)	3 ml	15 ml	2 x 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 HS** Add 15 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 12 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution LS** Add 60 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 96-9.8 % ethanol to each bottle and mix thoroughly. Keep the bottle always firmly closed!

Components not included in the kit

- DNase I; optional
- Reaction tubes
- Ethanol (70 %, 96–99.8 %)

NOTE

Use only absolute/pure ethanol, NO methylated or denatured alcohol!

7. Product specifications

1. Starting material:
   Different kinds of plant material (up to 100 mg)

2. Time for isolation:
   Approximately 30 minutes after homogenization

3. Typical yield:
   - Depending on the kind and initial amount of the starting material
   - Up to 70 µg

4. Binding capacity:
   Approximately: 100 µg RNA

8. GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Lysis Solution RL	Guanidinium thiocyanate 25–50 %	 *Danger*	302, 314, 412	101, 102, 103, 260,303+361+353, 305+351+338, 310, 405, 501
Lysis Solution PL	Guanidinium chloride 25–50 %	 *Warning*	302, 315, 319	101, 102, 103, 280, 264, 501, 305+351+338, 301+312, 332+313,
Washing Solution HS (conc.)	Guanidinium thiocyanate 50–100 %	 *Danger*	302, 314, 412	101, 102, 103, 260, 310, 405, 501, 303+361+353, 305+351+338,

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.
315	Causes skin irritation.
319	Causes serious eye irritation.
412	Harmful to aquatic life with long lasting effects.

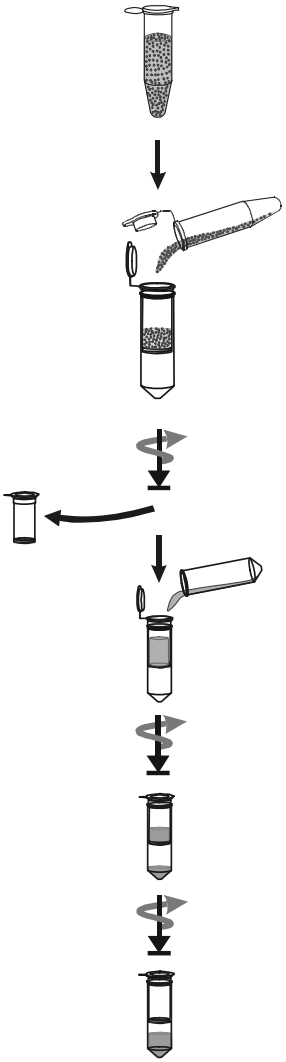
8.2 Precaution phrases

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.
264	Wash thoroughly after handling.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
310	Immediately call a POISON CENTER/doctor.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
301+312	IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.
332+313	If skin irritation occurs: Get medical advice/attention.
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.

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 RNA extraction



Homogenization and lysis of the starting material

Selective removing of gDNA by Spin Filter D

Selective binding of RNA onto Spin Filter R

Washing of the bound RNA

Elution of RNA

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: RNA extraction from plant material (up to 100 mg)

IMPORTANT
Please note that up to 100 mg of plant material can be processed.

1. Homogenization of starting material

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

For the homogenization of plant 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 or grind the starting material with sand.

A. Homogenization of the plant 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 µl Lysis Solution RL** or **450 µl Lysis Solution PL**.

NOTE
The yield of extracted RNA depends on the type of Lysis Solution used. Most plant material can be processed with **Lysis Solution RL**. Some kinds of plant material should be processed with **Lysis Solution PL**. Please start the extraction process with **Lysis Solution RL**. In case of low yield or no yield please use the second **Lysis Solution PL**.

3. Homogenize the sample.
4. Transfer the homogenized sample into a 1.5 ml reaction tube and place the sample under **Lysis Solution RL** or **Lysis Solution PL** for longer storage at -22 °C to -18 °C or use the sample immediately for isolation of total RNA following the protocol from step 2.

B. Disruption of the plant 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 µl Lysis Solution RL** or **450 µl Lysis Solution PL** and incubate the sample for appropriate time for a further lysis under continuous shaking.

NOTE
The yield of extracted RNA depends on the type of Lysis Solution used. Most plant material can be processed with Lysis Solution RL. Some kinds of plant material should be processed with Lysis Solution PL. Please start the extraction process with Lysis Solution RL. In case of low yield or no yield please use the second Lysis Solution PL.

4. Finally place the sample under **Lysis Solution RL** or **Lysis Solution PL** for longer storage at -22 °C to -18 °C or use the sample immediately for isolation of total RNA following protocol step 2.

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 11,000 x g (~11,000 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.

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 sometimes up and down. Transfer the sample onto the Spin Filter R. Centrifuge at 11,000 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 and place the Spin Filter R into a new Receiver Tube.

4. Open the Spin Filter R and add **500 µl Washing Solution HS**, close the cap and centrifuge at 11,000 x g (~11,000 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 **650 µl Washing Solution LS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.

6. Open the Spin Filter R and add **650 µl Washing Solution LS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter R into a new Receiver Tube.
7. Centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
8. 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 11,000 x g (~11,000 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. Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient disruption or homogenization	After lysis centrifuge lysate to pellet unlysed material 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 the starting material. 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, have been performed quickly.
RNase contaminations of solutions, Receiver Tubes etc.	Use sterile, RNase-free filter tips. Before every preparation clean up the pipet, 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 Solutions for salt precipitates. If there are any precipitates dissolve these precipitates by careful warming.

14. Related products

Name	Amount	Order No.
Products for PCR & Gel Electrophoresis		
innuSPEED Lysis Tubes P	50 tubes	845-CS-1020050
	100 tubes	845-CS-1020100
	250 tubes	845-CS-1020250
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 (12,5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
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

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