

Instructions for Use

Life Science Kits & Assays



innuPREP Plant RNA Kit

Order No.:

845-KS-2060010 10 reactions
845-KS-2060050 50 reactions
845-KS-2060250 250 reactions

Publication No.: HB_KS-2060_e_211021

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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 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 before 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 and the kit

For easy reference and orientation, the manual and labels use 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 before 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. IST Innuscreen GmbH 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.

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 on GHS classification please and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com

3. Storage conditions

The kit is shipped at ambient temperature.

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 IST Innuscreen GmbH 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 IST Innuscreen GmbH products, please do not hesitate to contact us.

For technical support or further information in Germany please dial +49 30 9489 3380. 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 (→ “Product specifications“ p. 8). Since the performance characteristics of IST Innuscreen GmbH kits have just been validated for the application described above, the user is responsible for the validation of the performance of IST Innuscreen GmbH kits using other protocols than those described below. IST Innuscreen GmbH 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 IST Innuscreen GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

NOTE

The kit is for research use only!

6. Kit components

6.1 Included kit components

	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

6.2 Components not included in the kit

- 1.5 ml tubes
- 96–99.8 % ethanol (non denatured or methylated)
- DNase I; optional

7. Product specifications

1. Starting material:
 - Up to 100 mg fresh, frozen or dried plant material
 - 120 - 180 mg wet weight (if material is very wet/ contains more water)

NOTE

Avoid freezing and thawing of starting material. If plant tissue will not be used immediately after harvesting, it can be stored in liquid nitrogen, lyophilized/dried or frozen. Fresh material can be kept at 4 °C to 8 °C for 24 hours but should be frozen at -22 °C to -18 °C or for longer storage at -80 °C for later processing. Ground tissue powder can also be stored at -80 °C. Alternatively, tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15 °C to 30 °C). To ensure DNA quality, samples should be completely dried within 24 hours of collection.

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

8. Initial steps before starting

- Add the indicated amount of absolute ethanol to **Washing Solution HS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-2060010	Add 3 ml ethanol to 3 ml Washing Solution HS (conc.).
845-KS-2060050	Add 15 ml ethanol to 15 ml Washing Solution HS (conc.).
845-KS-2060250	Add 70 ml ethanol to 70 ml Washing Solution HS (conc.).

- Add the indicated amount of absolute ethanol to **Washing Solution LS (conc.)**, mix thoroughly and store as described above. Always keep the bottle firmly closed.

845-KS-2060010	Add 12 ml ethanol to 3 ml Washing Solution LS (conc.).
845-KS-2060050	Add 60 ml ethanol to 15 ml Washing Solution LS (conc.).
845-KS-2060250	Add 160 ml ethanol to 40 ml Washing Solution LS (conc.).

- Centrifugation steps should be carried out at room temperature.

9. 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.

10. Protocol: RNA extraction from plant material

NOTE

For the homogenization of plant sample it is possible to use commercially available rotor-stator homogenizer or bead mills (e. g. SpeedMill PLUS from Analytik Jena GmbH). It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the plant sample to a fine powder or grind the starting material with sand.

10.1 Homogenization of plant sample

10.1.1 Homogenization using a rotor-stator homogenizer

NOTE

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

1. Transfer weighed amount of fresh or frozen starting material in a suitable reaction vessel for the homogenizer.
 2. Add 450 μ l Lysis Solution RL or 450 μ l Lysis Solution PL.
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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.

10.1.2 Disruption by using mortar and pestle

1. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine 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.

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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.
 5. 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.

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6. 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.

7. Discard the Receiver Tube with filtrate and place the Spin Filter R into a new Receiver Tube.
 8. 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.
 9. 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.
 10. 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.
 11. Centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
 12. 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.
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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.

11. 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. Checkup Washing Solutions for salt precipitates. If there are any precipitates dissolve these precipitates by careful warming.

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