Instructions for Use
Life Science Kits & Assays

innuSOLV RNA Reagent

Innuscreen GmbH

Order No.:

845-SB-2090010 10 ml
845-SB-2090100 100 ml

Publication No.: HB_SB-2090_e_220413

Manufacturer and Distributor:

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1 Introduction

1.1 Intended use

innuSOLV RNA Reagent is a reagent for the efficient isolation of total RNA from a wide variety of starting materials (e.g. tissue samples, cells, bacterial cells, plants, etc.) as well as from different amount of the starting materials. The extraction method is based on a particularly time-saving single-step liquid phase separation.

innuSOLV RNA Reagent contains a mixture of phenol and guanidine thiocyanate in mono-phase solution. After the addition of chloroform and subsequent centrifugation, the homogenate is separated into three phases:

- a colored lower organic phase
- a whitish interphase and
- an upper colorless aqueous phase.

The RNA is provided in the upper aqueous phase. From this aqueous phase the RNA is precipitated by the consecutively addition of an alcohol. The RNA extraction by using the innuSOLV Reagent could be finished within 1 hour. Thereby the extracted RNA is un-degraded and a high-quality nucleic acid and can be used for a multitude of downstream applications as northern analyses, cDNA synthesis, RT-PCR reactions, Dot-Blot hybridizations, Poly(A)+ selections, in vitro translations, cloning and RNase assays.

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	**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.
	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.
	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 and the kit" p. 3).
- Working steps are numbered.

2 Safety precautions

ATTENTION!
The innuSOLV RNA Reagent contains phenol and guanidine thiocyanate, which are harmful to health. 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 for at least 15 minutes. Afterwards adjourn yourself under medical care immediately

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.

WARNING
All reagents have to be handled under an exhaust hood 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 on GHS classification and the Safety Data Sheet (SDS) please contact sds.innu@ist-ag.com

3 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 hours or more before use. Autoclaving will not inactivate RNase activity 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 followed by autoclaving or heating to 100 °C for 15 minutes 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 water.
- 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.

4 Storage conditions

The innuSOLV RNA Reagent should be stored dry and protected from light at 4 °C and is stable until the expiration date printed on the label.

5 Functional testing and technical assistance

The IST Innuscreen GmbH guarantees the correct function of the kit for applications as described in the manual. This kit has been produced 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 innuSOLV RNA Reagent please do not hesitate to contact us. For technical support or further information in Germany please contact info.innu@ist-ag.com. For other countries please contact your local distributor.

6 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). Since the performance characteristics of our kits have not been validated for any specific application. 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!

7 Kit components

7.1 Included kit components

	Σ 10	Σ 100
REF	845-SB-2090010	845-SB-2090100
RNA Reagent	10 ml	100 ml
Manual	1	1

7.2 Components not included in the kit

- Chloroform
- Isopropanol
- 70 % ethanol (non denatured or methylated)
- Polypropylene centrifuge tubes (centrifugation with phenol @ 12.000 x g)
- RNase-free water

8 Product specifications

1. Starting material

- Tissue samples (100 mg)
- Monolayer cells
- Cell suspensions (animal, plant, yeast or bacterial cells, 5 x 10^6)

2. Time for isolation

Approximately 60 minutes

3. Typical yield

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

9 Initial steps before starting

- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting materials

10 Protocol for isolation of RNA from different types of starting material

10.1. Homogenization of the starting material

A. Tissue samples

Add **2 ml innuSOLV RNA Reagent** to **100 mg** of the **tissue sample** and homogenize the starting material.

NOTE
To optimize the lysis it is recommended to use a glass, a Teflon or an electrical homogenizer.

The sample volume should not be more than 10 % of the used volume of the innuSOLV RNA Reagent.

B. Monolayer cells

Add **1 ml innuSOLV RNA Reagent** directly to a 3.5 ml cell culture petri dish. Lyse the cells by pipetting up and down several times.

NOTE
The required volume of **innuSOLV RNA Reagent** does not depend on the number of cells, but on the size of the Petri dish (as a rule: 1 ml per 10 cm^2)

An insufficient volume of the **innuSOLV RNA Reagent** can cause a contamination with DNA.

C. Cell suspensions

Pellet cells (max. 5 x 10^6) by centrifugation, add **1 ml innuSOLV RNA Reagent** to the pellet and resuspend the sample.

NOTE
A wash step prior to cell lysis increases RNA degradation and should be avoided.

In certain cases, if yeast or bacterial cells are used, an additional homogenization or enzymatic pre-treatment could be necessary

10.2. Extraction of the RNA

1. Incubate the sample at room temperature for 5 minutes.
2. Add **chloroform (0.2 ml** per added **1 ml** of the **innuSOLV RNA Reagent)** and mix the sample by vortexing for 10 sec.
3. Incubate the sample on ice for 3 – 10 min.

NOTE
An incubation at room temperature is also possible but can cause a potential bad phase separation.

4. Centrifuge the sample at 12.000 x g (10.000 rpm) and 4 °C for 5 min. to separate the phases into:
   - A red colored lower organic phase
   - A white colored inter phase and
   - An upper colorless aqueous phase

   The RNA is enriched in the upper aqueous phase, whereas the DNA and the proteins are provided in the inter phase and the phenol phase. The aqueous phase is around 60 % of the total sample volume.

NOTE
The used chloroform should be free of additives, like isoamyl alcohol.

5. Transfer the aqueous phase carefully into a new centrifuge tube.

NOTE
A carry over of parts of the inter phase has to be avoided necessarily to prevent a contamination of the final RNA with DNA.

6. Add an equal volume of isopropanol, incubate the sample at 4 °C for 15 min and centrifuge at max. speed and 4 °C for 10 min.

NOTE
The consistence of the RNA precipitate is like a gel and should be at the lower side of the centrifuge tube.

7. Remove the supernatant carefully and wash the pellet two times using **1 ml of 70 % ethanol**.

8. Centrifuge the sample at max. speed for 10 min.

9. Dry the RNA pellet shortly by exposure to air or using a light vacuum.

NOTE
The completely drying of the pellet will downgrade the solubility of the RNA and should be avoided.

ATTENTION
Don't dry the RNA by vacuum centrifugation!

10. Solve the RNA by repeated pipetting up and down in deionized formamide, RNase-free water or 0.5 % SDS.

NOTE
The heating of the RNA to 55 – 60 °C will upgrade the solubility of the RNA. To avoid a contamination with RNase, the water or SDS solution should be pre-treated with diethyl pyrocarbonate (DEPC). It is also recommended to use commercially available RNase-free water.

11 Remarks

1. To avoid any RNase contaminations, it is recommended to wear gloves during the whole extraction and to use only RNase-free solutions and devices.

2. If only a low amount of RNA is expected (< 10 µg), it is recommended to add 70 µg glycogen per used 1 ml of the innuSOLV RNA Reagent as a carrier for the precipitation.

3. After homogenization and before the addition of chloroform, the samples could be stored at −70 °C for several month. After washing the RNA precipitate could be stored for 1 – 3 weeks at 4 °C under 75 % ethanol or for 1 year at −20 °C.

4. For the RNA extraction using the innuSOLV RNA Reagent, also table top centrifuges could be used if a max. speed of 2.600 x g is possible. Therefore the centrifuge times from step 2 and 3 have to be prolonged to 30 – 60 min.

5. If samples with a high percentage of proteins, polysaccharides, lipids or other contents are processed, an additional washing step is recommended. Before the separation of the phases using chloroform is done, unsoluble parts could be removed by centrifugation at max. speed and 4 °C for 10 min. The RNA is located in the supernatant, whereas the pellet contains the polysaccharides, extra cellular parts of the membrane and high molecular DNA's. If samples from high-fat tissues are used, a foamy fat layer occurs, which should be removed. The clear supernatant, which contains the RNA has to be transferred to a new centrifuge tube and the chloroform extraction can be performed as described above.

12 Troubleshooting

Problem / probable cause	Comments and suggestions
Low amount of extracted RNA	
Insufficient homogenization or lysis	Homogenize or lyse the starting material completely.
Insufficient dissolution of the RNA pellet	Solve the RNA pellet completely
Ratio A260/A280 too low	
Insufficient homogenization volume	Homogenize the sample in a bigger volume and incubate the sample at room temperature.
Contamination with phenol phase	Transfer the aqueous phase carefully.
Insufficient dissolution of the RNA pellet	Solve the RNA pellet completely.
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 during the preparation	Use sterile, RNase-free filter tips. Before every preparation clean up the pipette, the devices and the working place. Always wear gloves!
DNA contamination	
Insufficient homogenization volume	Homogenize the sample in a bigger volume. Samples may show no organic solutions, e. g. ethanol, DMSO, concentrated buffers or an alkaline pH-value.

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