Instructions for Use
Life Science Kits & Assays

innuSOLV RNA Reagent

analytikjena
An Endress+Hauser Company

Order No.:

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

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1 Safety precautions

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.

Warning!
All reagents have to be handled under an exhaust hood only!

2 Storage conditions

The innuSOLV RNA Reagent should be stored dry and protected from light at 4 °C and is stable for at least 6 months under these conditions.

3 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual.

We reserve the right to change or modify our products to enhance there performance and design. If you have any questions or problems regarding any aspects of the innuSOLV RNA Reagent or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 (0) 36 41 / 77 94 00. For other countries please contact your local distributor.

4 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. 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!
The usage for diagnostic purpose or as pharmaceutical products, as well as the application to humans or animals is not permitted!

5 Product description

Modificated guanidine isothiocyanate / phenol method for the extraction of RNA

5.1 Intended use

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

5.2 Extraction principle

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

- A colored lower organic phase
- A whitely inter phase and
- A 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.

6 Product specifications

1. Starting material:

- Tissue samples (100 mg)
- Monolayer cells
- Cell suspensions (animal, plant, yeast or bacterial cells; 5×10^{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.

7 Recommended steps before starting

- Centrifugation steps should be performed at room temperature
- Avoid freezing and thawing of starting materials

8 Components not included

- Chloroform
- Isopropanol
- Ethanol (70 %)
- Polypropylene centrifuge tubes (centrifugation with phenol @ 12.000 x g)
- RNase-free water

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 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.

10 Protocol: RNA extraction different kinds of starting materials

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 (e.g. SpeedMill P12).

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 cm cell culture petri dish. Lyse the cells by repeated up and down pipetting.

Note: The needed volume of the innuSOLV RNA Reagent is not depending on the number of cells, but on the size of the petri dish (as a general 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 re-suspend the sample.

Note: A washing step before the lysis of the cells takes place, enhances the degradation of RNA an should be avoid.

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

2. Phase separation

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 to a potential bad phase separation.

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
- A 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.

3. RNA precipitation

1. 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.

2. 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.

4. Washing of the RNA

1. Remove the supernatant carefully and wash the pellet two times using **1 ml, 70 % ethanol**
2. Centrifuge the sample at max. speed for 10 min.

5. Solving of the RNA

1. 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.

Don't dry the RNA by vacuum centrifugation!

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

   Note: The heating of the RNA to 55 – 60 % 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 commercial 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, un-soluble 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
Amount of RNA is too less	
• 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 to less	
• 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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