NOYA-RNAsol

RNA extraction reagent

Catalog number: N-1121

« Research Use Only »

RNAsol reagent is designated for the isolation of total RNA from cultured cells and other biological fluids. It separates RNA in a single – step liquid phase. The kit is guanidium based solution according modified protocol of Sacchi and Chomzynski. Its main advantages are less time consuming and more sensitive for low abundant transcript.

Precautions

RNAsol isolation reagent consists of phenol and guanidium which both have toxic characteristics. Avoid contact this solution with acidic condition and also be aware in contact with skin and other mucosa. Good lab circulation as prerequisite is highly recommended.

1- Work under fume Hood.
2- Wear gloves, Lab Coat, appropriate eye protection.
3- Avoid breathing vapor.
4- Chloroform (store at 2-8˚C). Keep from heat, avoid breathing vapor.
5- Isopropanol (store at 2-8˚C). Keep from heat, Hazardous in case of eye contact (irritant), of ingestion, of inhalation.
6- RNA-Binder (store at -20˚C).
7- Ethanol 75% (store at 2-8˚C).
8- Elution buffer (store at 2-8˚C).

Applications

Isolation of **total RNA** from cultured cells, Plasma Cell Suspension and other biological fluids.

Procedure

1- for each 250µl of plasma or cell suspension (cell density 10^5-10^6cell/ml), Add 1000 µl RNAsol reagent to a 1.5ml screw-cap microtube at 15 -25˚C.
2-Homogenized the suspension by passing it several time through pipetting and next with syringe 20G at least 30 times.
Note: The homogenates sample can be stored at -60C or below for at least one month before is used in step 3 (Separation Phase).
3- Incubate the homogenized sample for 5 min at room Temperature to ensure the complete dissociation of nucleoprotein complex.
4- Add 200µl chloroform to each micro tube.
5- Cap securely & shake it vigorously by hand for 15 sec.
6- Incubate at Room temperature for 2-15 min.
7- Separate the solution in three phases, centrifuge the microtube at 12000g for 15min at 2C -8C.
Note: in the case of working with cell suspension it is more conclusive to repeat chloroform extraction twice. For this reason, transfer upper aqueous phase to new 1.5ml microtube and add equal volume of chloroform and repeat steps 5-7.

8- Transfer the upper aqueous phase (which is approximately 60% of the volume of whole reagent) to a new 1.5ml microtube.
Optional: first add 5µl RNA Binder as a carrier to assist RNA precipitation at bottom of microtube.
9- Add 500µl isopropanol to the aqueous phase (for precipitation the RNA from the colorless aqueous phase).
10- Invert the tube several times to mix it thoroughly or vortex it (5-10 sec).
11- Incubate for 10 minute at 15-25C to complete precipitation of RNA.
12- Centrifuge it at 12000g for 10 min at 2-8˚C.
13- Discard the supernatant.
14- Add 1ml Nuclease free 75% ethanol to each microtube.
Note: prepare Nuclease free 75 % ethanol with DEPC ultrapure water.
15- Centrifuge it at 7500g for 5 min at 2- 8˚C.
16- Discard the supernatant.
17- Repeat the step 14-16.
18- Remove the exceed ethanol from the RNA pellet by Air Drying or Placing it under vacuum for 5-10 min.
Caution: Don't dry the RNA pellet by centrifugation under vacuum, don't let dry completely as a dry pellet will be less soluble
19- Add 20µl Elution buffer (TE pH 7:50 or nuclease free water) to the pellet RNA.
20- Dissolve the pellet RNA by passing the solution through a pipette tip several times or vortex it 5-10 sec.
21- Incubate it for 10 min at 55-60˚C.

The RNA may be used immediately for amplification, or maybe stored at -80˚C

Notes

- **FOR RESEARCH USE ONLY NOT FOR HUMAN OR DIAGNOSTIC USE.**
- Please observe General laboratory precaution and utilize safety while using this kit.

Related Products

- RNA-binder
- DEPC- treated water
- Nuclease free water