RNA-Bee - RNA ISOLATION REAGENT

Catalog No: CS-104B - 100 ml / CS-105B - 200 ml / CS-501B - 500 ml
Storage: Store at 2 - 8 C. STABILITY: RNA-Bee is stable at 2 - 8 C for at least one year from date of purchase.

PRODUCT DESCRIPTION

RNA-Bee is a complete and ready-to-use reagent for isolation of total RNA from samples of human, animal, plant, bacterial and viral origin. RNA-Bee is the improved version of the single-step method of RNA isolation (1). The improved RNA-Bee provides a fast and highly reliable method for isolating pure and undegraded RNA from a large variety of biological samples.

RNA-Bee and the single-step method are subjects of the US patent 4,843,155. RNA-Bee is a monophase solution containing phenol and quanidine thiocyanate. A biological sample is homogenized or lysed in RNA-Bee and the homogenate/lysate is separated into aqueous and organic phase by the addition of chloroform. The subsequent centrifugation efficiently removes DNA and proteins from the aqueous phase containing RNA. The undegraded, pure RNA is obtained from the aqueous phase by the isopropanol precipitation, washing with ethanol and solubilization in an appropriate solution. The entire isolation procedure can be completed in 1 hour. The isolated RNA is appropriate for Northern blotting, poly A + selection, RT-PCR, and other molecular biology techniques.

SPECIAL HANDLING PRECAUTIONS

RNA-Bee contains a poison (phenol) and an irritant (guanidine thiocyanate). CAUSES BURNS. Can be fatal. When working with RNA-Bee, use gloves and eye protection (face shield, safety goggles). Do not get on skin or clothing. Avoid breathing fumes. Read the warning note on the container and MSDS. In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek medical attention.

I. PROTOCOL FOR RNA ISOLATION

Reagents required but not supplied: chloroform isopropanol, and ethanol.
We recommend the use of disposable polypropylene tubes. The tubes should be tested to ensure integrity during centrifugation at 12,000g with RNA-Bee and chloroform.
The protocol describes isolation of RNA with 1 ml of RNA-Bee using the following steps:
1. HOMOGENIZATION 1 ml RNA-Bee + 50 mg tissue or 5 x 10^6 cells
2. PHASE SEPARATION homogenate + 0.2 ml chloroform
3. RNA PRECIPITATION aqueous phase + 0.5 ml isopropanol
4. RNA WASH 1 ml 75% ethanol
5. RNA SOLUBILIZATION Water, 0.5 % SDS or buffer
All steps can be carried out at room temperature unless otherwise stated.

1. HOMOGENIZATION

A. TISSUES. Homogenize tissue samples in RNA-Bee (1 ml / 50 mg tissue) using a glass-glass, glass-teflon, or Polytron homogenizer. The sample volume should not exceed 10% of the RNA-Bee volume.
B. CELLS. Cells grown in monolayer should be lysed directly in the culture dish by the addition of RNA-Bee. Use at least 1 ml of the reagent for a 3.5 cm petri dish. Pass the lysate through a pipette several

times to ensure lysis. Cells grown in suspension should be sedimented first and then lysed by the addition of RNA-Bee. Add at least 0.2 ml of RNA-Bee per 10^6 cells and lyse by repeated pipetting.

2. PHASE SEPARATION

Add 0.2 ml chloroform per 1 ml of RNA-Bee, cap the tube and shake vigorously for 15 - 30 seconds. Store the sample on ice (or at least 4 C) for 5 minutes. Centrifuge the homogenate at 12,000g for 15 minutes at 4 C. Following centrifugation, the sample forms the lower blue phenol-chloroform phase, interphase, and the upper colorless aqueous phase. RNA remains exclusively in the aqueous phase whereas DNA and proteins are in the interphase and organic phase. The volume of the aqueous phase is about 50% of the initial volume of RNA-Bee plus sample volume. Chloroform should not contain isoamyl alcohol or any other additives.

3. RNA PRECIPITATION

Transfer the aqueous phase to a clean tube, add 0.5 ml of isopropanol, and store the sample for 5-10 minutes at room temperature. Centrifuge at 12,000g for 5 minutes at 4 - 25 C. RNA precipitate (often not visible before centrifugation) forms a white-yellow pellet at the bottom of the tube.

4. RNA WASH

Remove the supernatant and washs the RNA pellet once with 75% ethanol, shaking or votexing to dislodge the pellet from the side of the tube. Centrifuge for 5 minutes at 7,500g at 4 - 25 C. Use at least 1 ml of ethanol solution per 1 ml of RNA-Bee used for the initial homogenization. An additional wash with 75% ethanol improves 260/280 ratio and might be necessary to use the isolated RNA in enzymatic assays.

5. RNA SOLUBILIZATION

At the end of the procedure, briefly air-dry the RNA pellet (5 - 10 minutes). It is important not to let the RNA pellet dry completely, as this greatly decreases its solubility. Do not dry RNA by centrifugation under vacuum. Dissolve the RNA in water, 0.5% SDS or buffer by passing the solution through a pipette ip and/or incubating for 10 - 15 minutes at 55 - 60 C. Tubes, water or solutions used for RNA solubilization should be made RNase-free by diethyl pyrocarbonate (DEPC) treatment. The final preparation of RNA has a 260/280 ratio 1.6 - 1.9.

II. COMMENTS

1. Isolation of RNA from a small amount of tissue (1-10 mg) can be accomplished by homogenizing the sample in 0.8 ml of RNA-Bee. Transfer the homogenate to an Eppendorf tube, add 160□l of chloroform, and store the sample for 5 minutes at 4 C. Centrifuge for 15 minutes at 4 C, collect the aqueous phase and precipitate the RNA with 0.4 ml of isopropanol for 30 minutes or overnight at 4 C. Centrifuge RNA precipitate at 10,000g for 10 minutes at 4 - 25 C. Wash the pellet once with 75% ethanol.
2. Following isopropanol addition, store the sample overnight at 4 C in case the procedure has to be interrupted at this step.
3. Hands and dust are a significant source of RNase contamination. Use gloves and keep tubes clean.
4. Some commercial SDS preparations have an acidic pH. Adjust pH to 6.5 - 7.5 if necessary.

III. REFERENCES

1. P. Chomczynski and N. Sacchi, Anal. Biochem. 162, 156-159 (1987).

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