Guanidine Isothiocyanate Solution
[4 M guanidine isothiocyanate, 50 mM Tris-HCl (pH 7.5), 25 mM EDTA]

Cat. No.: 15577-018 **Size: 500 ml**
Store at 4°C.

Description

Guanidine isothiocyanate is a strong protein denaturant. In a highly concentrated guanidine isothiocyanate solution, many proteins unfold and separate into their constituent polypeptides. Guanidine Isothiocyanate is widely used to purify nucleic acids from cell extracts. Due to its properties as an RNase inhibitor, Guanidine Isothiocyanate Solution is especially useful for the isolation of RNAs from sources rich in RNase, such as pancreas tissue (1-3).

CAUTION: This product is considered hazardous. Please consult the Material Safety Data Sheet for health and safety information.

Overview of mRNA purification

The most important aspect of any RNA isolation procedure is the inactivation of endogenous ribonucleases (RNases) as quickly as possible to prevent degradation of the RNA and loss of sequence information. One of the most effective methods for cellular disruption uses high concentrations of the chaotropic salt guanidine isothiocyanate in the presence of 2-mercaptoethanol to rapidly denature RNases in aqueous solution (1-3). The denatured RNases are physically separated from the RNA by repeated ethanol precipitation of the RNA from chaotropic salt solutions under conditions where the protein remains in the supernatant fraction following centrifugation.

Isolation of total RNA is the first step in the isolation of mRNA. Most eukaryotic mRNAs contain poly(A) tails at their 3´ termini ranging in size from 50 to 200 nucleotides (4,5). The poly(A) tails can be used to physically select mRNA from a total RNA population by affinity chromatography (6,7). The RNA is passed over a chromatography matrix (cellulose is commonly used) containing immobilized deoxythymidine oligomers (oligo dT) in the presence of 0.3 to 0.5 M NaCl to promote hybridization of the poly(A) tails to the oligo dT. After the column is washed to remove unbound nucleic acids, the mRNA is eluted by destabilizing the hybrids of oligo dT and poly(A). This generally is done by removing the NaCl, and occasionally by raising the temperature. This can yield an RNA preparation that contains >90% mRNA.

Protocols

The following procedure is an example of the use of Guanidine Isothiocyanate Solution in a complete procedure for the isolation of total RNA and mRNA from tissues or cells. This protocol is designed for a 1-g sample of tissue or wet-weight of packed cells. The volumes may be scaled proportionally for different sample masses.

Isolation of Total RNA

1. Prepare 25 ml of guanidine isothiocyanate/2-mercaptoethanol (GuSCN/ME) solution by adding 2 ml of 2-mercaptoethanol to 23 ml of Guanidine Isothiocyanate Solution in a 50 ml sterile, disposable polypropylene centrifuge tube. Mix by inversion and chill on ice for 10 min.
2. Add 15 ml of the GuSCN/ME solution to a 50 ml centrifuge tube and place on ice for 10 min.
3. While the GuSCN/ME solution is cooling on ice, chill a power homogenizer probe in 25 ml of ice-cold autoclaved distilled water for 5 min.
4. Transfer the cell pellet or tissue sample to the ice-cold GuSCN/ME solution in the 50 ml centrifuge tube and immediately homogenize it until it is completely disrupted (20 to 40 s at maximum speed).

5. Add 4.5 ml of ethanol (0.3 volume), mix by inversion several times, and centrifuge the suspension at 16,000 x *g* for 5 min at 4°C.
6. During the centrifugation, clean the homogenizer by running it in several changes of autoclaved distilled water.
7. Using a sterile pipette, remove the supernatant and the floating protein film that may form after centrifugation by careful aspiration **from the top down**. The supernatant fraction is highly enriched for the denatured RNases and must be removed carefully to avoid bringing the floating film into contact with the RNA pellet.
8. Add 7.5 ml of the ice-cold GuSCN/ME solution to the RNA pellet and dissolve it by homogenization for 10 s at 70% to 100% maximum speed.
9. Centrifuge the solution at 16,000 x *g* for 3 min at 4°C. Transfer the supernatant fraction to a 15 ml sterile, disposable polypropylene centrifuge tube and discard the pellet.
10. Add 188 µl of 1 M acetic acid (0.025 volume) and 5.6 ml of ethanol (0.75 volume). Mix by inversion several times and chill at -20°C for 10 min.
11. Centrifuge at 7,000 x *g* for 10 min at 4°C and discard the supernatant fraction.
12. Prepare 25 ml of guanidine hydrochloride/2-mercaptoethanol (GuHCl/ME) solution by adding 20 µl of 2-mercaptoethanol to 25 ml of Guanidine Hydrochloride Solution [6 M guanidine hydrochloride (pH 7.5), 25 mM EDTA] in a 50 ml sterile, disposable polypropylene centrifuge tube. Mix by inversion and chill on ice for 10 min.
13. Add 10 ml of the GuHCl/ME solution to the RNA pellet from step 11 and disrupt the pellet by trituration (repeated expulsion of the pellet from a sterile pipette). Be patient! Not all of the particulate material will go into solution, but the particulate size must be reduced to maximize RNA yield. It is not necessary to remove the particulates before proceeding.
14. Add 0.5 ml of 1 M acetic acid (0.05 volume) and 5 ml of ethanol (0.5 volume). Mix by inversion several times and chill at -20°C for 10 min.
15. Centrifuge at 7,000 x *g* for 10 min at 4°C and discard the supernatant fraction.
16. Add 7 ml of GuHCl/ME solution to the RNA pellet and dissolve by trituration with a sterile pipette. As in step 13, not all of the pellet will go into solution.
17. Add 0.35 ml of 1 M acetic acid (0.05 volume) and 3.5 ml of ethanol (0.5 volume). Mix by inversion several times and chill at -20°C for 10 min.
18. Centrifuge at 7,000 x *g* for 10 min at 4°C and discard the supernatant fraction.
19. Add 5 ml of GuHCl/ME solution to the RNA pellet and dissolve by trituration with a sterile pipette. As in step 13, not all of the pellet will go into solution.
20. Add 0.25 ml of 1 M acetic acid (0.05 volume) and 2.5 ml of ethanol (0.5 volume). Mix by inversion several times and chill at -20°C for 10 min.
21. Centrifuge at 7,000 x *g* for 10 min at 4°C and discard the supernatant fraction.
22. **CAUTION:** It is extremely important to observe all precautions designed to prevent introduction of RNase into the sample from this point until the end of the procedure.
23. Add 3 ml of room-temperature binding buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.3 M NaCl, 0.1% (w/v) SDS] to the RNA pellet and dissolve by trituration using a sterile pipette.
24. Add 6 ml (2 volumes) of ethanol. Mix by inversion several times and chill at -20°C for 10 min.
25. Centrifuge at 7,000 x *g* for 10 min at 4°C and discard the supernatant fraction.

Isolation of mRNA from Total RNA

1. Pack 100 mg of oligo(dT) Cellulose (Cat. No. 15940-026) into the bottom of a disposable plastic column. Clamp the column to a ringstand.
2. Load 1 ml of 0.1 M NaOH onto the column and let it drain completely.
3. Equilibrate the column with 4 ml of binding buffer in 1 ml aliquots.
4. Load 1 ml of binding buffer onto the column and let about 0.5 ml pass through. The column now is ready to use. If you wish to store the column, cap the ends of the column securely and store at 4°C.
5. Add 3 ml of binding buffer to the RNA pellet and dissolve by trituration using a sterile pipette.
6. Heat the RNA solution in a 70°C water bath for 5 min and chill on ice for 5 min. If the SDS in the buffer precipitates, warm it to room temperature only and swirl it until the SDS goes back into solution.
7. If there are any particulates that do not go into solution, remove them by centrifugation at approximately 1,000 x *g* for 5 min at room temperature and retain the supernatant fraction. A small, table-top clinical centrifuge is sufficient for this purpose.
8. Dilute a 10 µl aliquot from the clarified solution with 990 µl of binding buffer. Read the A_{260} of this solution with the spectrophotometer blanked against binding buffer. Calculate the amount of RNA:

   $$\text{Total RNA (mg)} = \frac{A_{260} \times 100 \times 3 \text{ ml}}{25\ A_{260}/\text{mg/ml}}$$

   where A_{260} is the absorbance of the solution measured with the spectrophotometer at 260 nm, 100 is the dilution factor, 3 ml is the total volume of the RNA solution, 25 A_{260}/mg/ml is the conversion factor relating absorbance to concentration. If the amount of RNA exceeds 20 mg, run the oligo-dT cellulose column more than once to prevent overloading.
9. Load the dissolved RNA (20 mg) onto the column under gravity flow and wash with 4 ml of binding buffer to elute non-messenger RNA.
10. Elute the mRNA with 1.5 ml of elution buffer (10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% (w/v) SDS) and collect the eluate as one fraction in a 15 ml sterile, disposable centrifuge tube.
11. Re-equilibrate the oligo(dT) cellulose column with 4 ml of binding buffer.
12. Heat the RNA from step 10 in a 70°C water bath for 5 min and chill in an ice-water bath for 5 min.
13. Place the RNA solution at room temperature for 20 min, then add 90 µl of 5 M NaCl. I**mmediately load the RNA onto the column and wash it with 4 ml of binding buffer.**
14. Elute the mRNA with 1.5 ml of elution buffer and collect the eluate as one fraction in a 15 ml sterile, disposable centrifuge tube.
15. Add 90 µl of 5 M NaCl and 3 ml of ethanol to the RNA. Place the tube at -20°C overnight, or until needed.
16. The oligo(dT) column should now be rewashed, as in step 2, followed by washing with autoclaved distilled water until the pH of the effluent is neutral, using pH indicator strips to monitor the approximate pH. Re-equilibrate the oligo(dT) cellulose column with 4 ml of binding buffer before reusing. This column may be used to purify mRNA from a total of 5 g of sample before it should be discarded.
17. Centrifuge sample from step 15 at 7,000 x *g* for 20 min at 4°C and remove the supernatant fraction.
18. Carefully add 1 ml of ethanol to the tube and centrifuge at 7,000 x *g* for 2 min at 4°C.
19. Remove the supernatant fraction and dry the pellet at room temperature until the ethanol has evaporated completely, approximately 30 min. This can be hastened by using a heating block at 50°C for approximately 10 min.
20. Dissolve the pellet in a minimal volume of DEPC-treated 1 mM EDTA (pH 7.5) solution (5 to 50 µl is recommended) and store at -70°C if available; if not, store at -20°C.

References

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