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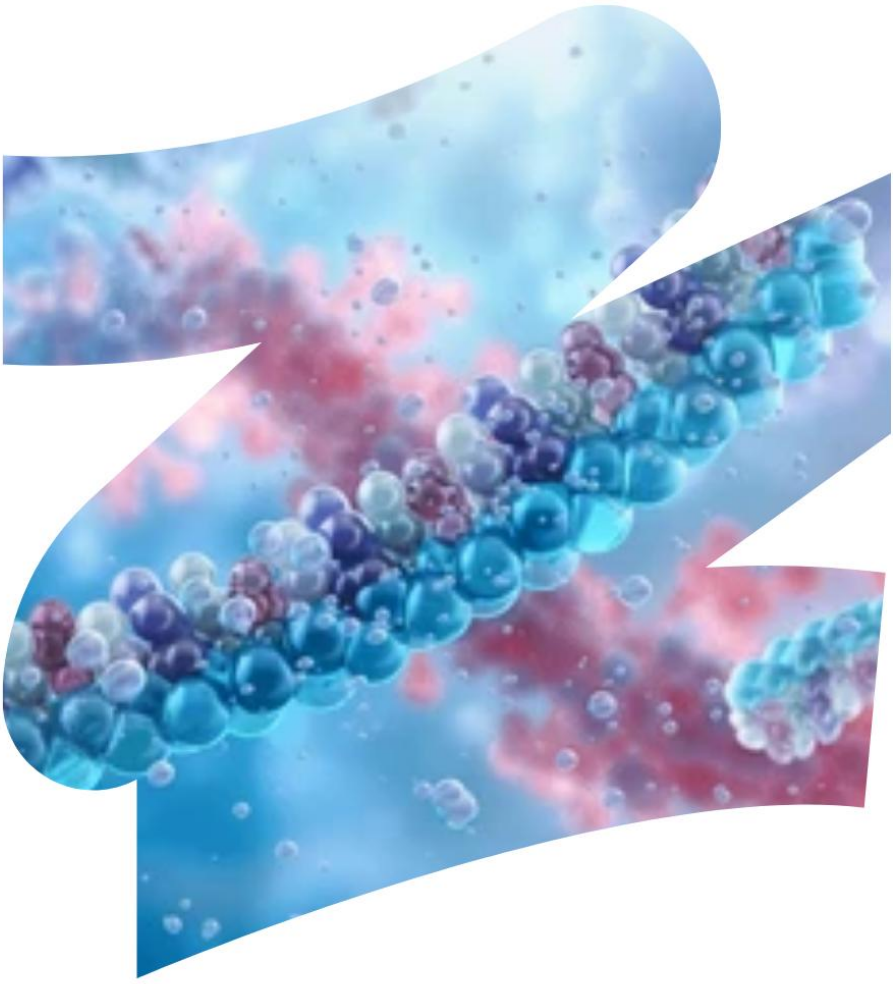

SN Total RNA Extraction Teaching Kit

Solution Based

Innovation Speaks

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SN Total RNA Extraction Teaching Kit

Product Code:
Number of experiments that can be performed:
10 Duration of Experiment

Protocol: 1 hour
Agarose Gel Electrophoresis: 1 hour

Storage Instructions:

- The kit is stable for 6 months from the date of manufacture
- Store 5X RNA Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

Isolation and purification of total RNA from animal tissue.

Introduction:

SN Total RNA Extraction Teaching Kit is designed for rapid extraction and purification of total RNA for Northern analysis, Poly A^+ RNA selection, Primer extension, RNase and S1 nuclease protection assays, RT-PCR, Differential display, Expression-array and Expression-chip analysis. Total RNA is extracted using RNA X-Press reagent. This is one of the most effective method for isolating total RNA and can be completed in only 1 hour starting with fresh tissue and cells.

Principle:

SN Total RNA Extraction Teaching Kit is designed for rapid purification of RNA from different samples using RNA X-Press reagent. This product which is a mixture of guanidine thiocyanate and phenol in a mono-phase solution which effectively dissolves RNA. After adding Chloroform and centrifuging, the mixture separates into 3 phases: an aqueous phase containing the RNA, the interphase containing DNA and an organic phase containing proteins. RNA is precipitated using isopropyl alcohol. Impurities are removed using ethanol and pure RNA is resuspended in RNase-Free Water. 1 ml of RNA-XPress Reagent is sufficient to isolate RNA from 50-100 mg of tissue. This advanced RNA isolation procedure is an improvement to the single-step RNA isolation using phenol and guanidine isothiocyanate developed by Chomczynski and Sacchi.

Kit Contents:

This kit can be used to perform total RNA extraction from animal tissue.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity	Storage
			10 expts	
1	DS0044	RNA - Xpress™ Reagent	12 ml	R T
2	MB063	Isopropanol	6 ml	R T
3	DS0042	RNase Free Water*	1.5 ml	R T
4	ML048	5X RNA Loading Buffer	0.03 ml	2-8°C
5	ML050	10X MOPS Electrophoresis Buffer	600 ml	R T
6	MB059	Formaldehyde 37%	130 ml	R T
7	MB002	Agarose	7.2 g	R T
8	PW1139	Collection Tubes, Polypropylene (2.0 ml)	20 Nos.	R T

* RNase Free Water provided in the kit is for preparation of 75% Ethanol and for elution of RNA.

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker
Reagents: Ethidium bromide (10 mg/ml), Ethanol, Chloroform, Diethyl pyrocarbonate, RNase free water for gel electrophoresis

Other requirements: Electrophoresis apparatus, UV Transilluminator, Heating block or Water Bath, Vortex Mixer, Tabletop Micro centrifuge (with rotor for 2.0 ml tubes), Micropipettes, Tips with aerosol barrier, Adhesive tape

Storage:

SN Total RNA Extraction Teaching Kit can be stored at room temperature (15-25°C) for up to 6 months from the date of manufacture without showing any reduction in performance. On receipt, store 5X RNA Loading Buffer at 2-8°C. Other reagents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Thoroughly mix the reagents. Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.
3. Ensure the use of only clean & dry DNase, RNase free eppendorf tubes and tips for the procedure.
4. **75% Ethanol for one experiment:** Add 0.75 ml of ethanol (96-100%) to 0.25 ml of RNase free water.
5. Set the centrifuge at 4°C before starting the experiment.

Precautions to be taken while handling RNA:

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.
2. Collection tubes, tips, pipettes, electrophoresis unit etc to be used for the experiment must be UV treated for 15-20 minutes.
3. Use sterile, disposable plasticware and micropipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.
4. Non-disposable plasticwares should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.
5. Glassware used for RNA work should be cleaned with detergent, thoroughly rinsed, and oven baked at 240°C for at least four hours before use. Alternatively glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.
6. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
7. Solutions (water and other solutions) should be treated with 0.1% DEPC.

Procedure:

1. **Sample Preparation**

   Homogenize 50 mg of animal tissue in 1 ml of RNA X-Press Reagent using autoclaved motor and pestle. Transfer the mixture to a 2.0 ml collection tube.

2. **Phase separation**

a. Incubate the homogenized sample for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes.

b. Add 200 µl of Chloroform (not provided) per ml of RNA-Xpress Reagent used. Cover the sample tightly, Vortex vigorously for 15 seconds and allow the tube to stand for 10 minutes at room temperature (15-25°C).

c. Centrifuge the resulting mixture at 13,000 rpm for 15 minutes at 4°C. Following centrifugation, the mixture separates into lower organic phase (containing protein), an interphase (containing cell debris and DNA) and upper aqueous phase containing RNA.

NOTE: The Chloroform used for phase separation should not contain Isoamyl alcohol and other additives.

3. **RNA Precipitation**

a. Transfer the aqueous phase containing RNA to a fresh tube and add 500 µl of Isopropanol. Allow the sample to stand for 10 minutes at room temperature (15-25°C).

b. Centrifuge at 13,000 rpm for 10 minutes at 4°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. **RNA Wash**

Remove the supernatant and wash the RNA pellet by adding 1 ml of 75% ethanol. Pipette gently to resuspend the pellet and then centrifuge at 10,500 rpm for 5 minutes at 4°C.

NOTE: Be careful while discarding / decanting the supernatant as the pellet might also get discarded along with the supernatant.

5. **RNA Elution**

Briefly dry the RNA pellet for 10 minutes by air-drying. Resuspend the pellet with 50 µl of RNase-Free Water.

NOTE: Do not let the RNA pellet dry completely, as this will greatly decrease its solubility. Do not dry the RNA pellet by centrifugation under vacuum.

Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturation of RNA.

Agarose Gel Electrophoresis:

Preparation of 1X MOPS Electrophoresis Buffer: To prepare 500 ml of 1X MOPS Electrophoresis buffer, add 50 ml of 10X MOPS Electrophoresis Buffer to 440 ml of RNase free water and add 10 ml of Formaldehyde (37%). Mix well before use.

Preparation of agarose gel: To prepare 50 ml of 1.2 % agarose solution, mix 5 ml of 10X MOPS Electrophoresis Buffer with 45 ml of autoclaved deionized water in a glass beaker or flask. To this add 0.6 g of agarose. Heat the mixture in a microwave, burner or hot plate by swirling the glass beaker/ flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow solution to cool to about 55-60°C. Add 0.9 ml of 37% Formaldehyde and mix well. Immediately add 0.5 µl Ethidium Bromide, mix well and pour the gel solution into the gel tray sealed on both sides with adhesive tape. Allow the gel to solidify for about 30 minutes at room temperature (15-25°C).

NOTE: Before running the gel, equilibrate it in 1X MOPS Electrophoresis Buffer for at least 30 minutes.

Loading of the RNA samples: To prepare sample for electrophoresis, add 2 µl of 5X RNA Loading Buffer per 10 µl of RNA sample. Load onto the equilibrated MOPS gel.

Electrophoresis: Connect power cord to the electrophoretic power supply according to the conventions: Red- Anode and Black- Cathode. Electrophorese at 100 volts and 70 mAmp until dye markers have migrated an appropriate distance, depending on the size of RNA to be visualized.

Quantitation of RNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use RNase-Free Water to dilute the samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The concentration of RNA is calculated by the following formula:

Concentration of RNA sample (µg/ml) = 40 x A_{260} x dilution factor.

Observation and Result:

Perform Agarose Gel Electrophoresis. Visualize the RNA bands using UV Transilluminator and calculate the yield and purity using Spectrophotometer.

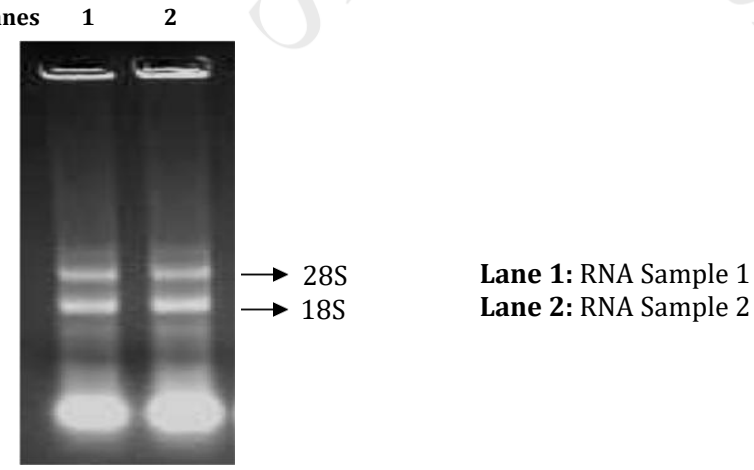

Fig 1: Gel image of extracted RNA from animal tissue

Table 2: Absorbance of the extracted RNA at 260 nm and 280 nm

Sample	Dilution Factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration (µg/ml)
1					
2					

Calculate the concentration of isolated RNA using the following formula:

Concentration of RNA sample (µg/ml) = 40 x A_{260} x dilution factor.

Interpretation:

The lanes 1 and 2 demonstrate that total RNA has been obtained when electrophoresed on agarose gel. If the entire procedure is not carried out properly, RNA will be degraded and will appear as a smear. An absorbance of
1.0 at 260 nm corresponds to approximately 40 µg/ml of RNA. The A_{260} -A_{320} /A_{280} -A_{320} ratio should be 1.8 –2.1.

Troubleshooting Guide:

Sr.No.	Problem	Possible Cause	Solution
1	Low yield of RNA	Incomplete homogenization or lysis of samples	No particulate matter should remain in the tube. Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes
		RNA pellet was not dissolved completely	To facilitate dissolution, mix by repeated pipetting with a micropipette at 55-60°C for 10-15 minutes
2	Degraded RNA	The isolated RNA samples have been stored at -20°C	The samples should be stored at -80°C as specified in the procedure
		Aqueous solution or tubes used for procedure may contain RNases	Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross contamination with RNases from shared equipments
3	DNA contamination	Presence of DNA	Samples should be digested with RNase-Free DNase

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