Experiment time: 2025-06-20, 18:00 - 2025-06-20, 24:00

RNA Extraction

I. Objective of the experiment

The T24 cell lines and low, medium and high drug-resistant T24-RC48 cell lines iin two six-well plates were treated with TRIzol reagent to extract RNA, thus providing the material foundation for subsequent qPCR experiments.

II. Experimental content

2.1 Experimental design

Total RNA was extracted by lysing cells in TRIzol reagent firstly. After centrifugation using chloroform for phase separation, collect the supernatant and add isopropanol to precipitate RNA. Wash with 75% ethanol, dry the sample, and dissolve it in RNase-free water. Adherence to standard protocols ensures the yield of high-quality RNA for subsequent molecular biology studies. Improper handling, such as RNA degradation, may compromise experimental accuracy and reliability.

2.2 Sample types

T24 wild-type cell line, low/medium/high drug resistance T24-RC48 cell line.

2.3 Measurement principle

The TRIzol method for RNA extraction operates through the synergistic action of phenol and guanidine thiocyanate in the reagent system. Guanidine thiocyanate acts as a strong denaturant to rapidly lyse cells, releasing RNA while inhibiting endogenous RNase activity to prevent degradation. Phenol facilitates protein denaturation and precipitation. After adding chloroform, centrifugation creates a three-phase system: an upper aqueous phase (containing RNA), a middle phase (denatured proteins), and a lower organic phase (containing DNA and lipids). Under acidic conditions, RNA selectively dissolves in the aqueous phase. Subsequent isopropanol precipitation separates RNA, followed by ethanol washing to remove salts, yielding high-purity RNA. This method achieves efficient RNA isolation and purification through chemical denaturation, phase separation, and selective precipitation.

III. Materials and reagents

3.1 Materials

Pipette tips and 15ml centrifuge tubes were purchased from Axygen

Micropipettes: Purchased from Eppendorf

Ice bucket

2ml EP tubes

Human bladder transitional cell carcinoma cell line (T24)

Low/medium/high resistance T24-RC48 cell lines

3.2 Reagents

PBS: Purchased from Gibco, USA

TRIzol reagent: purchased from Thermo Fisher Scientific

Isopropanol and 75% ethanol were purchased from Sensi Chemical

Chloroform

IV. Experimental instruments

Fume hood

Vortex mixer

Benchtop low-speed centrifuge at room temperature: Eppendorf

Spectrophotometer: purchased from Thermo Fisher Scientific

V. Experimental steps

5.1 The T24 cell line and the low, medium and high drug-resistant T24-RC48 cell lines were inoculated into two six-well plates, with two wells per cell line.

5.2 Take two six-well plates out of the 37℃ constant temperature box and place them in a fume hood and on ice.

5.3 Transfer 1ml PBS with a pipette into the eight holes of the experimental group, gently shake and clean, and repeat twice.

5.4 Add 500μl TRIzol reagent to each well to lyse the cells, and pipette repeatedly until the lysate is homogeneous and no visible cell clumps remain. Then suck the positive, low, medium and high drug-resistant cells into four 2ml EP tubes labeled as "positive", "low", "medium" and "high".

5.5 Add chloroform (tri-chloromethane) to each of the four EP tubes at 1/5 of the volume of

TRIzol reagent for extraction. Vortex vigorously for 15 seconds to ensure complete emulsification.

5.6 After shaking, the solution was left at room temperature for 5min and then centrifuged at 12,000×g for 15 minutes at 4°C. At this time, the solution was obviously divided into three layers: the upper layer was water phase, the middle layer was protein and the lower layer was organic phase.

5.7 Carefully aspirate the upper aqueous phase, 200μl *2/ per tube (aspirate part, do not touch the middle protein layer) and place it in a new EP tube.

5.8 Add an equal volume of pre-cooled isopropanol to the EP tube, invert and mix, and leave at room temperature for 15min to precipitate.

5.9 12000g 4℃ Centrifuge for 10min, discard the supernatant, add 1ml of pre-cooled 75% ethanol to wash the precipitate twice.

5.10 12000g 4℃ Centrifuge for 5min, discard the supernatant, and dry the centrifuge tube at room temperature to allow the residual ethanol to volatilize.

5.11 The dried pellet was resuspended in 20μL of RNase-free water and left on ice.

5.12 RNA concentration and purity were quantified using a spectrophotometer.

VI. Photo of experimental operation

Figure 1 After centrifugation, the precipitate was observed after adding isopropanol

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VII. Experimental results

The concentration of the extracted RNA was > 700 ng/μL, and the A260/A280 ratios were between 1.9 and 2.1.

#	样品名称	ng/μL	A260/A280
1	样品 1	719.7	1.91
2	样品 2	1302.8	1.93
3	样品 3	1015.1	1.91
4	样品 4	1115.4	1.92
5	样品 5	967.1	1.93

Figure 2 Results of RNA quantification

VIII. Results analysis

The experiment was successful in achieving its objective of extracting RNA from cells. The standardized protocol demonstrated minimal operational errors: The treated cell samples showed uniform lysis characteristics with no visible clumps or residual impurities. Centrifugation yielded clear three-phase separation—aqueous phase at the top, protein-rich middle layer, and organic phase at the bottom—fulfilling the expected lysis efficiency. Subsequent extraction procedures—including lysis, extraction, precipitation, and washing—yielded RNA samples with optimal concentration and high purity, establishing a solid foundation for subsequent reverse transcription.

However, several areas for improvement were identified during the experiment. For instance, while the TRIzol reagent dosage and centrifugation parameters followed standard protocols, further optimization could be achieved through subsequent RNA quality testing to enhance extraction efficiency and product quality. These observations will guide further optimization of the experimental protocol.