Up Plus RNA extraction Kit

Catalog No.: abx098093

Size: 100 rxns

Storage: Store the Up Plus reagent at 4 °C in the dark for 12 months. Store the other kit components at room temperature (15-25 °C) for 12 months.

Application: For the isolation of total RNA from cells and tissues. This kit can be used to rapidly isolate RNA from many different species including humans, animals, plants and bacteria in one hour.

Introduction

RNA extraction is the purification of RNA from biological samples. This procedure is complicated by the ubiquitous presence of ribonuclease enzymes in cells and tissues, which can rapidly degrade RNA. Obtaining high-quality RNA is critical for performing many molecular techniques including reverse transcription real-time PCR (RT-qPCR), transcriptome analysis using next-generation sequencing, array analysis, digital PCR, northern analysis, and cDNA library construction.

Principle of the Assay

Up Plus RNA extraction Kit is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The addition of chloroform to the sample, and the following centrifugation separate the solution into an upper colorless aqueous phase (containing RNA), intermediate phase and a lower pink organic phase. RNA is specifically bound to silica-based spin column. The Up Plus RNA extraction Kit provides powerful lysis and easy column based purification. This kit also has powerful lysis capability, giving a higher purity and RNA yield.

Kit components

1. Up Plus Reagent: 100 ml
2. Clean Buffer 9 (CB9): 110 ml
3. Wash Buffer 9 (WB9): 24 ml
4. RNase-free water: 40 ml
5. RNA spin columns with collection tubes: 100
6. RNase-free tubes: 100

Material Required But Not Provided

1. 96% - 100% Ethanol
2. Chloroform or 4-Bromoanisole
3. 1x PBS
4. Pipettes and pipette tips
5. Centrifuge
6. Homogenizer
7. Liquid Nitrogen

Protocol

A. Sample Preparation

- **Adherent cells:** Wash culture dish once with 1x PBS. Add 1 ml of Up Plus reagent for every 10 cm^2 culture dish. Incubate for a few minutes at room temperature. Pass the cell lysate through a pipette tip several times and, for adherent cells, detach cells using a cell scraper. Transfer the cell lysate into a microcentrifuge tube, and pipette up and down to disperse any visible precipitate. Incubate at room temperature for 5 minutes.
- **Bacterial culture and suspension cells:** Transfer bacterial culture or suspension cells (including culture dish) to a microcentrifuge tube. Centrifuge samples at 8000 x g at 4 ^oC for 2 minutes and discard the supernatant. Add 1 ml of Up Plus reagent for every 2 x 10^9 bacteria or 1 x 10^7 suspension cells. Pipette lysates up and down several times until there are no visible precipitates present. Incubate at room temperature for 5 minutes.
- **Animal tissues and plant materials:** Weigh out the frozen sample and quickly transfer into a mortar with liquid nitrogen. Grind thoroughly to a powder and use more liquid nitrogen if required. Incomplete grinding can affect RNA yield and quality. Transfer the tissue powder to a microcentrifuge tube. Add 1 ml of Up Plus reagent for every 50-100 mg of tissue. Homogenize tissue samples with a homogenizer and repeatedly pipetting up and down. Incubate at room temperature for 5 minutes.

B. Reagent Preparation

- **Wash Buffer 9 (WB9):** Add 96 ml of 96% - 100% ethanol to WB9 (24 ml) before use. The total volume of this reagent should be 120 ml.

C. Assay Procedure

1. Add 0.2 ml chloroform (or 50 µl 4-Bromoanisole) for every ml of Up Plus reagent. Shake the tube vigorously for 30 seconds and incubate at room temperature for 3 minutes.
2. Centrifuge samples at 10 000 x g at 4 ^oC for 15 minutes. The mixture separates into a lower pink organic phase, interphase and a colorless upper aqueous phase containing the RNA.
3. Transfer the colorless, upper phase to a fresh RNase-free tube. Avoid addition of the interphase or lower phase to prevent DNA contamination. Add 96-100% ethanol at an equal volume to the aqueous upper phase liquid. Mix gently by inverting the tube.
4. Transfer the resulting solution and precipitates together to a spin column. Centrifuge at 12 000 x g at room temperature (RT) for 30 seconds. Discard the flow-through.
5. Add 500 µl of CB9 to the spin column. Centrifuge at 12 000 x g at room temperature (RT) for 30 seconds. Discard the flow through. Perform this step twice.
6. Add 500 µl of WB9 to the spin column. Centrifuge at 12 000 x g at RT for 30 seconds. Discard the flow through. Perform this step twice.
7. Centrifuge at 12 000 x g at RT for 2 minutes to completely remove any remaining ethanol. Air-dry the column matrix for several minutes.
8. Place the spin column into a clean 1.5 ml RNase-free tube. Add 50 µl - 200 µl RNase-free water into the spin column matrix. Incubate at RT for 1 minute.
9. Centrifuge at 12 000 x g at RT for 1 minute to elute RNA.
10. Store the isolated RNA at -80 ^oC.