Blood RNA Isolation Kit (Catalog# TBS6002)

Single-Step Method

DESCRIPTION

Isolation of high quality RNA is an important first step in gene expression studies such as RT-PCR, qRT-PCR and array analysis. TBS's Blood RNA Isolation Kit is designed to isolate total RNA from PBMC. Under acidic conditionsof reagent, total RNA remains in the upper aqueous phase, while most of DNA and proteins remain either in the interphase or in the lower organic phase. Total RNA is then recovered by precipitation with isopropanol and can be used for several applications. This kit can significantly improve the quantity and quality of RNA.

APPLICATIONS

Direct Assays: total RNA isolation from PBMC.
The yield of total RNA from 1 x10^7 PBMC should range from 50 µg to 80 µg. The A260/A280 ratio of the isolated RNA should be above 1.8.

KEY FEATURES

Simple and convenient: There is no special equipment need.
High yield rate: This method gets higher yield rate of RNA than spin column method.
Versatility: multiple RNAs include microRNA.

KIT CONTENTS (50 samples)

Reagent: 50 ml
RNase-free Water: 10 ml
Ficoll: 500 ml

Storage conditions. The kit is shipped at room temperature. Store the reagent at 4°C. Shelf life: 12 months after receipt.

REQUIRED MATERIALS NOT PROVIDED WITH THEKIT

Chloroform, Isopropanol, Ethanol, 1.5 ml tubes

PROCEDURES

Isolating PBMC

1. Obtain 10 ml whole blood (1-2x10^6 PBMC per ml of whole blood). 1:1 dilute blood with PBS (free Ca/Mg^{++}) in 50 ml tube.
2. Underlay this carefully with 10 mL of Ficoll.
3. Spin at 1600 rpm for 30 min, brake off (since you have a gradient i.e. cells are in layers).
4. Resultant layers are approximately from top to bottom: Plasma – platelets -- PBMC – Ficoll – red blood cells (with granulocytes).
5. Carefully aspirate the buffy coat with PBMC's, andtransfer to one new 50 mL tube.
6. Add enough PBS to the PBMC's to make up 50 mL. Spin at 1200 rpm for 10 min, brake on (*cell pelleting)*.
7. Decant the supernatant, loosen pellet, and wash once again in PBS.

Isolating total RNA

1. Add 1 ml RNA Isolation Reagent per 1x10^7 PBMC and resuspend the lysate at least ten times with 1-ml pipette tip.
2. Add 0.2 ml chloroform and shake vigorously by hand or vertex for 10s
3. Cool the samples on ice for 15 min. Centrifuge for 15 min at 12,000g at 4°C.
4. Transfer carefully the upper aqueous phase (mostly RNA, 0.5ml) to a clean tube.
5. Add 0.5ml (1 vol.) of isopropanol, mix well and incubate the sample for at least 1 h at -20°C.
6. Centrifuge at 12,000g at 4°C for 20 min to precipitate the RNA. Discard the supernatant carefully.
7. Wash the RNA pellet with 1 ml of 75% ethanol, centrifuge at 10,000g at 4°C for 5 min. Discard the supernatant and repeat washonce.
8. Air-dry RNA pellet for 5-10 min (never let the RNA pellet air-dry completely).
9. Dissolve the RNA pellet in 100-200 µl of RNase-free water (if need, Incubate RNA 10–15 min at 60 °C to ensure complete solubilization).
10. Assess concentration and quality of RNA with spectrophotometric readings at wavelengthsof 260nm and 280nm (Pure preparations of RNA have an A_{260}/A_{280} ratio of between 1.8 and 2.0). Store the RNA samples at -80°C.

REFERENCES

1. Chomczynski P & Sacchi N. Single-stepmethod of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction.Anal. Biochem. 162: 156-159 (1987).

2. Chomczynski P & Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nature Protocols, 1: 581-585(2006)