High-Q™ Spin Column TIARIZOL™ PLUS RNA Purification Kit

Ordering info

TBK0243, 3 reactions (sample)

TBK0244. 50 reactions

TBK0245. 200 reactions

Description

High-Q™ Spin Column TIARIZOL™ PLUS RNA Purification Kit is an ideal kit to obtain high quality RNA from a wide variety of samples, including cultured cells, animal and plant tissues, blood, bacteria and yeast. The kit combines the effective lysis of TIARIZOL™ Reagent with the efficient purification system based on silica High-Q™ RNA spin columns to provide ultrapure total RNA. The procedure includes sequential steps: lysis, followed by silica adsorption and washing steps. It is not necessary a phase separation, or post-purification steps. The kit allows to make high amounts of RNA which are very clean due to the column step.

Features

- High yield and purity (A260/A280 ~1.8).
- RNA suitable for downstream applications.

Applications

- cDNA library
- Northern blotting
- Nuclease protection assay
- RT-PCR
- *In vitro* translation

Quality Control

Functionally tested with cultured cells.

Kit Components

Components	TBK0244	TBK0245
TIARIZOL™ Reagent	50 mL	3 x 60 mL
High-Q™ RNA Spin Column	50 units	200 units
DNase I (5 U/µL)	250 µL	2x 500 µL
10x DNase-I Buffer	1.5 mL	2 x 1.5 mL
WRNA-1 Buffer	20 mL[a]	70 mL[b]
WRNA-2 Buffer	12 mL[c]	45 mL[d]
Water, nuclease free	15 mL	25 mL

Order Info Kit Components: *TIARIZOL™ Reagent (TBR0102) | High-Q™ RNA Spin Column with Collection Tubes (TBM0012) | WRNA-1 Buffer (TBB0544) | WRNA-2 Buffer (TBB0545) | Water, nuclease free (TBB0302).*

Before its use

[a] Add 12 mL of Absolute Ethanol

[b] Add 42mL of Absolute Ethanol

[c] Add 48 mL of Absolute Ethanol

[d] Add 180 mL of Absolute Ethanol

Storage

Store the kit at 25°C.

Store DNase-I and 10x DNase-I Buffer at -20° C.

Store TIARIZOL™ Reagent at 4°C. Protect from light.

Reagents Required (not supplied)

- Ethanol (CAS 64-17-5).
- 1.5 mL microcentrifuge tubes
- 2 mL tubes

Also available:

TIARIZOL™ Reagent (TBR0100)

PROTOCOL

I. SAMPLE PREPARATION

Cultured cells: Harvest cells by centrifugation and decant the supernatant. Resuspend the pellet with the supernatant remaining. Add 0.8 mL of TIARIZOL™ Reagent per 5–10 × 10^6 cells. Lyse cells by repetitive pipetting up and down.

Bacterial Cells: From a stationary culture harvest bacterial cells by centrifugation. Resuspend the pellet with 200 µL Tris-HCl 10 mM, pH=7.5. Add 10 µL Lysozyme (50 mg/mL) and mix by vortex. Incubate at 37°C for 30 minutes. Then add 0.8 mL of TIARIZOL™ Reagent and homogenize well.

Yeast: From a stationary culture harvest yeast cells by centrifugation. Disruption may require a homogenizer. Then add 0.8 mL of TIARIZOL™ Reagent and homogenize well.

Plant Tissue: Use up to 50 mg of fresh vegetable material and up 20 mg of dried material. Plant material can be homogenized with liquid nitrogen using a mortar and a pestle or using any commercially available equipment for homogenization. Add 0.8 mL of TIARIZOL™ Reagent to homogenized material and mix well.

Animal tissue: Use up to 5 mg of animal tissue. Homogenization can be done with liquid nitrogen using a mortar and a pestle or using any commercially available equipment for homogenization. Add 0.8 mL of TIARIZOL™ Reagent to homogenized material. Mix well.

After addition of TIARIZOL™ Reagent, incubate 5 minutes at room temperature. Samples with TIARIZOL™ Reagent can be stored at -80°C until RNA extraction procedure.

II. RNA EXTRACTION

1. If the samples were stored at -80°C, thaw and homogenize them.
   A homogeneous sample is essential before RNA Purification steps.
2. Centrifuge at 13,000 g for 5 minutes at 4°C and transfer the supernatant to a 2 mL clean tube.
3. Add **1 volume of absolute ethanol** and mix.
4. Transfer **700 µL of the mixture** in the High-Q™ RNA Spin Column reservoir coupled to a Collection Tube.
5. Centrifuge at 10,000g for 1 minute. Discard the flow-through.
6. Repeat steps 4-5 for the remaining mixture obtained in step 3.
7. To dry silica matrix, centrifuge at 10,000g for 1 minute.
8. Add **50 µL DNase Mixture** (5 µL DNase-I + 45 µL 10x DNase-I Buffer) in the center of High-Q™ RNA Spin Column.
9. Incubate for 15 minutes at room temperature (15-25°C).
10. Add **500 µL WRNA-1 Buffer** (✓) and centrifuge at 10,000 g for 1 minute. Discard the flow-through and place the High-Q™ RNA Spin Column back into the Collection Tube.
    ✓ *Check Ethanol has been added.*
11. Add **700 µL WRNA-2 Buffer** (✓) and centrifuge at 10,000 g for 1 minute. Discard the flow-through.
    ✓ *Check Ethanol has been added.*

12. Place High-Q™ RNA Spin Column back in the Collection Tube and repeat step 11.
13. To dry silica matrix, centrifuge at 13,000g for 1 minute.
14. Place High-Q™ RNA spin column into a clean 1.5 mL microcentrifuge tube (RNase-free).
15. Carefully and without touching the matrix, add in the center of High-Q™ RNA Spin Column, **50-100 µL of molecular biology grade water** (nuclease free) prewarmed at 65°C.
16. Incubate at room temperature for 1-2 minutes.
17. Centrifuge for 1 minute at 13,000 g. RNA isolated is in the eluate. Discard High-Q™ RNA Spin Column.
18. Store at -80°C.