High-Q™-Spin-Column Total RNA Purification Kit

Ordering info

TBK0247-3, 3 reactions (sample) TBK0247-50, 50 reactions TBK0247-100, 100 reactions

Description

High-Q™-Spin-Column Total RNA Purification Kit is an easy silica-membrane-based system for RNA purification from a wide variety of sources (plant and animal tissues, bacteria cells and cell cultures). An optimized lysis buffer guarantees a good yield while the use of High-Q™ RNA Spin Columns allows a good quality RNA, suitable for downstream applications.

Features

- **Safety**, no phenol extraction.
- **High yield and purity** using the amount of sample recommended: A260/A280 ~2.0; A260/A230 ~2.0-2.2; RIN 7-9).
- Isolated **RNA is ready to use for downstream molecular biology applications**.

Applications

- Purification of RNA from different sources.
- RNA obtained is suitable for downstream molecular biology applications such as RT-PCR, RT-qPCR, Northern, cDNA library, nuclease protection assay, *in vitro* translation, etc.

Quality Control

RNA purified is checked by integrity (agarose gel electrophoresis), quantity and quality (A260/280 1.9-2.1).

Kit Components

Components	TBK0247-50	TBK0247-100
High-Q™ RNA Spin Columns (*green ring*)	50	100
Collection Tubes	50	100
Filter Spin Columns with Collection Tubes (*white ring*)	50	100
Total RNA Buffer-1	35 mL[a]	70 mL[a]
Total RNA Buffer-2	4 mL	10 mL
DNase I (5 U/µL)	300 µL	600 µL
DNase-I Buffer	2 x 1.5 mL	10 mL
Lysozyme	30 mg	2 x 30 mg
WRNA-1 Buffer	20 mL[b]	35 mL[d]
WRNA-2 Buffer	12 mL[c]	25 mL[e]
Water, nuclease free	5 mL	10 mL

Order Info Kit Components: *High-Q™ RNA Spin Columns (TBM0012) | Collection Tubes (TBM0020) | Total RNA Buffer-1 (TBB0583) | Total RNA Buffer-2 (TBB0584) | DNase-I (TBZ0320) | DNase Buffer (TBB0319) | Lysozyme (TBZ0312) | WRNA-1 Buffer (TBB0544) | WRNA-2 Buffer (TBB0545) | Water, nuclease free (TBB0302).*

¡Components for samples are ready to use!

Before its use:

[a] Add 10 µL β-mercaptoethanol per 1 mL Total RNA Buffer-1. Reducing agents such as DTT or TCEP may be use as alternative.

[b] Add 12 mL absolute ethanol and mix well.

[c] Add 48 mL absolute ethanol and mix well.

[d] Add 21 mL absolute ethanol and mix well

[e] Add 100 mL absolute ethanol and mix well.

Storage

Store the kit at 25°C and DNase-I at -20°C.

Material required (not supplied)

- 1.5 mL Microcentrifuge tubes (RNase free).
- Ethanol (CAS 64-17-5).
- β-mercaptoethanol (βME) (CAS 60-24-2), DTT (TBR0150) or TCEP (TBR0104).

PROTOCOL

I. SAMPLE PREPARATION

Plant Tissue: Grind up to 100 mg of fresh vegetable material and up 20 mg of dried material. Plant material can be frozen at -80°C before grind up, can be homogenized with liquid nitrogen using a mortar and a pestle or using any commercially available equipment for homogenization. Using a chilled spatula, transfer the resulting powder into a pre-cooled 1.5 mL RNase-free tube. Proceed with Module II.

Animal tissue: Use up to 10-20 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. Using a chilled spatula, transfer the resulting powder into a pre-cooled 1.5 mL RNase-free tube. Proceed with Module II.

Cultured cells: Harvest 10^6-10^7 cells by centrifugation and decant the supernatant. Resuspend the pellet with the supernatant remaining. Proceed with Module II.

Bacterial Cells: From a stationary culture harvest 10^9 bacterial cells by centrifugation. Discard the supernatant and 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. Proceed with Module II.

II. RNA PURIFICATION

Use gloves and change them frequently!

1. Add **600 µL Total RNA Buffer-1** and vortex vigorously to mix.
   Check βME has been added. All sample must be mixed with the buffer.
2. Apply the lysate into a Filter Spin Columns (*white ring*) placed into a Collection tube.
3. Centrifuge at 11,000 g for 1 min. Save the flow-through and transfer it to a new tube.
   Steps 2-3 are not necessary if you are working with bacterial or cultured cells.
4. Add **60 µL Total RNA Buffer-2** and vortex vigorously to mix.
   This step is not necessary if you are working with cultured cells.
5. Incubate at 50 °C for 5 minutes. Mix by inversion from time to time.
6. Centrifuge at 13,000 g for 5 minutes.
7. With a pipette, transfer the supernatant very carefully to another tube, avoiding cellular debris.
8. Add **0.5 volumes** of **absolute ethanol** (~ 250 µL). Mix by inversion.
9. Transfer up 700 µL mixture to a High-Q™ RNA Spin Column placed into a Collection Tube.
10. Centrifuge at 10,000 g for 1 minute. Remove the flow-through and place back the High-Q™ RNA Spin Column into a Collection Tube. If necessary, repeat steps 8 and 9 with the remaining mixture.
11. Centrifuge at 10,000 g for 1 minute to dry the column matrix.
12. Add **50 µL DNase Mixture** in the center of High-Q™ RNA Spin Column.
    ***DNase Mixture**: Mix with pipette 5 µL DNase-I + 45 µL 10x DNase-I Buffer. Avoid vortex.*
13. Incubate for 15 minutes at room temperature (15-25°C).

14. 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.*

15. Add **500 µL WRNA-2 Buffer** (✓).

✓ *Check Ethanol has been added.*

16. Centrifuge at 10,000 g for 1 minute. Discard flow-through. Place High-Q™ RNA Spin Column back in the Collection Tube and repeat from step 14.

17. To dry silica matrix, centrifuge at 10,000g for 1 minute.

18. Place High-Q™ RNA spin column into a clean 1.5-mL microcentrifuge tube.

19. Carefully and without touching the matrix, add in the center of High-Q™ RNA Spin Column, **50-100 µL Water** (nuclease free) prewarmed at 65°C.

20. Incubate at room temperature for 1-2 minutes.

21. Centrifuge for 1 minute at 13,000 g. RNA isolated is in the eluate. Discard High-Q™ RNA Spin Column.

22. Store at -80°C.