High-Q™ Spin Column Cultured Cell RNA Purification Kit

Ordering info

TBK0261, 3 reactions (sample)

TBK0262, 50 reactions

TBK0263, 100 reactions

Description

High-Q™-Spin-Column Cultured Cells RNA Purification Kit is an easy silica-membrane-based system for RNA purification from cultured cells. An optimized lysis buffer guarantees a good yield while the use of High-Q™ RNA Spin Columns allow a good quality RNA, suitable for downstream applications.

Features

- **Safety**, no phenol extraction, no ethanol precipitation.
- **High yield and purity,** 10-25 µg RNA with A260/A280 ~2.0; A260/A230 ~2.0-2.2.
- Isolated **RNA is ready to use for downstream molecular biology applications**.
- **Easy and fast protocol**.

Applications

- Purification of RNA from cultured cells.
- 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 ~2.0).

Kit Components

Components	TBK0262	TBK0263
High-Q™ RNA Spin Column with Collection Tubes	50	100
BCRNA-1 Buffer	25 mL[a]	50 mL[a]
DNase I (5 U/µL)	250 µL	500 µL
10x DNase-I Buffer	2 x 1.5 mL	10 mL
WRNA-1 Buffer	20 mL[b]	35 mL[c]
WRNA-2 Buffer	12 mL[d]	25 mL[e]
Water, nuclease free	5 mL	10 mL

Order Info Kit Components: *High-Q™ RNA Spin Columns with collection tubes (TBM0012) | BCRNA-1 Buffer (TBB0552) | DNase-I (TBZ0320) | 10x DNase Buffer (TBB0319) | 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 BCRNA-1 Buffer.

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

[c] Add 21 mL absolute ethanol and mix well

[d] Add 48 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 70%.
- β-mercaptoethanol (βME) (CAS 60-24-2).
- PBS 1x, pH=7.4.

PROTOCOL

1. Harvest the cells (10^6 – 10^7 cells) as usual and resuspend 1 x10^6 cells in 200 µL PBS 1x pH=7.4.
2. Add **600 µL BCRNA-1 Buffer** (✓) and mix vigorously by vortex for 30 seconds.
   ✓ *Check β-mercaptoethanol has been added. All cells must be mixed with the buffer.*
3. Add **600 µL ethanol 70%**. Mix by inversion.
4. Transfer up 700 µL mixture to a High-Q™ RNA Spin Column placed into a Collection Tube.
5. 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 4 and 5 with the remaining mixture.
6. Centrifuge at 10,000 g for 1 minute to dry the column matrix.
7. Add **50 µL DNase Mixture** in the center of High-Q™ RNA Spin Column.
   ***DNase Mixture**: Mix with a pipette 5 µL DNase-I + 45 µL 10x DNase-I Buffer. Avoid vortex.*
8. Incubate for 15 minutes at room temperature (15-25 °C).
9. 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.*
10. Add **500 µL WRNA-2 Buffer** (✓).
    ✓ *Check Ethanol has been added.*
11. Centrifuge at 10,000 g for 1 minute. Discard flow-through. Place High-Q™ RNA Spin Column back in the Collection Tube and repeat step 11.
12. To dry silica matrix, centrifuge at 10,000g for 1 minute.
13. Place High-Q™ RNA spin column into a clean 1.5-mL microcentrifuge tube.
14. 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.
15. Incubate at room temperature for 1-2 minutes.
16. Centrifuge for 1 minute at 13,000 g. RNA isolated is in the eluate. Discard High-Q™ RNA Spin Column.
17. Store at -80°C.