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

Ordering info

TBK0268-S, 3 reactions (sample)

TBK0268, 50 reactions

TBK0269, 100 reactions

Description

High-Q™-Spin-Column Tissue RNA Purification Kit is an easy silica-membrane-based system for RNA purification from a wide variety of animal tissues. 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:** up to 100 µg per sample (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 human and animal tissue (e.g. muscle, spleen, intestine, liver, heat, brain, rodent tail), insects, biopsy material.
- Purification of RNA from animal 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; A260/A230).

Kit Components

Components	TBK0268	TBK0269
High-Q™ RNA Spin Column with Collection Tubes	50	100
Tissue-RNA1 Buffer	35 mL[a]	60 mL[a]
Tissue-RNA2 Buffer	2 x 1.8 mL	10 mL
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[c]	35 mL[d]
WRNA-2 Buffer	12 mL[f]	25 mL[g]
Water, nuclease free	5 mL	10 mL

Order Info Kit Components: *High-Q™ RNA Spin Columns (TBM0012) | Tissue-RNA1 Buffer (TBB0581) | Tissue-RNA2 Buffer (TBB0582) | 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 Tissue-RNA1 Buffer.

[b] Add 6 mL absolute ethanol and mix well.

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

[d] Add 21 mL absolute ethanol and mix well

[e] Add 24 mL absolute ethanol and mix well.

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

[g] 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)

PROTOCOL

1. Grind up to 10-25 mg of tissue sample in liquid nitrogen using a mortar and a pestle. With a freeze spatula, collect the powder into a frozen 1.5 mL tube.
2. Add **600 µL Tissue-RNA1 Buffer** and mix vigorously by vortex.
   Check β-ME has been added. All material sample must be mixed with the buffer.
3. Add **60 µL Tissue-RNA2 Buffer** and mix vigorously by vortex.
4. Incubate at 50 °C for 5 minutes. Mix by inversion from time to time.
5. Centrifuge at 13,000 g for 5 minutes, at 4°C.
6. With a pipette, transfer the supernatant very carefully to another tube.
7. Add **0.5 volumes** of **absolute ethanol** (~ 300 µL). Mix by inversion.
8. Transfer up 700 µL mixture to a High-Q™ RNA Spin Column placed into a Collection Tube.
9. 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.
10. Centrifuge at 10,000 g for 1 minute to dry the column matrix.
11. Add **50 µL DNase Mixture** in the center of High-Q™ RNA Spin Column.
    ***DNase Mixture per sample**: Mix with pipette 5 µL DNase-I + 50 µL 10x DNase-I Buffer. Avoid vortex. It is recommended do a mix for all samples.*
12. Incubate for 15 minutes at room temperature (15-25°C).
13. 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.*
14. Add **500 µL WRNA-2 Buffer** (✓).
    ✓ *Check Ethanol has been added.*
15. 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 14.
16. To dry silica matrix, centrifuge at 10,000g for 1 minute.
17. Place High-Q™ RNA spin column into a clean 1.5-mL microcentrifuge tube.
18. 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.
19. Incubate at room temperature for 2 minutes.
20. Centrifuge for 1 minute at 13,000 g. RNA isolated is in the eluate. Discard High-Q™ RNA Spin Column.
21. Store at -80°C.