TIARIS

High-Q™ Spin Column Blood RNA Purification Kit

Ordering info

TBK0265, 3 reactions (sample)

TBK0266, 50 reactions

TBK0267, 100 reactions

Description

High-Q™-Spin-Column Blood RNA Purification Kit is an easy silica-membrane-based system for RNA purification from blood and cell cultures. 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 0.8-3 µg RNA from blood, 24-30 µg from culture cells.
- Isolated RNA is ready to use for downstream molecular biology applications.
- Suitable for blood and cell culture samples.

Applications

- Purification of RNA from blood.
- 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	TBK0266	TBK0267
High-Q™ RNA Spin Column with Collection Tubes	50	100
20x RBC Buffer	30 mL	60 mL
BBRNA-1 Buffer	35 mL[a]	70 mL[b]
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[e]	25 mL[f]
Water, nuclease free	5 mL	10 mL

Order Info Kit Components: High-Q™ RNA Spin Columns (TBM0012) | 20x RBC Buffer (TBB0399) | BBRNA-1 Buffer (TBB0553) | 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 35 µL βME and mix well. Valid for 1 month.

[b] Add 70 µL βME and mix well. Valid for 1 month.

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

[d] Add 21 mL absolute ethanol and mix well

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

[f] 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)

- 15 mL Centrifuge Tube (RNase free).
- 1.5 mL Microcentrifuge tubes (RNase free).
- Ethanol (CAS 64-17-5).
- β-mercaptoethanol (CAS 60-24-2).
- PBS 1x pH=7.4

For Research Use Only
© TIARIS Biosciences, 2023

PROTOCOL

1. In a 15 mL centrifuge tube, mix **1 volume of blood** (up 1.5 mL) in **5 volumes 1x RBC Buffer**.

   For mammalian cells cultured, resuspend 10^6 cells in 200 µL PBS 1x pH 7.4. Continue in step 7.

2. Incubate for 15 minutes on ice. During incubation, mix briefly 2 times by vortex.

   Solution has to become translucent as indication of cell lysis.

3. Centrifuge at 500g for 10 minutes at 4 °C. Discard the supernatant.
4. Add **2 volumes 1x RBC Buffer** considering the initial blood sample used.
5. Mix vigorously by vortex for 30 seconds.
6. Centrifuge at 500 g for 10 minutes at 4 °C. Discard the supernatant completely.

   Incomplete removal of the supernatant will interfere in the subsequent steps.

7. Add **BBRNA-1 Buffer** (✓) attending to the volume of initial blood sample used. For cells cultured use 600 µL.

V(Blood Sample)	V(BBRNA-1 Buffer)
≤ 500 µL	350 µL
500 µL – 1.5 mL	600 µL

   ✓ *Check β -mercaptoethanol has been added.*

8. Homogenize the mix by pipetting.
9. Transfer the mixture to a 1.5 mL microcentrifuge tube and centrifuge at 15,000 g, 5 minutes.
10. Transfer the supernatant to a fresh 1.5 mL microcentrifuge tube and add 1 volume of ethanol 70%. Mix by vortex.
11. Transfer up 700 µL mixture to a High-Q™ RNA Spin Column placed into a Collection Tube.
12. Centrifuge at 10,000 g, 1 minute. Remove the flow-through and place back the High-Q™ RNA Spin Column into the Collection Tube. If necessary, repeat steps 11 and 12 with the remaining mixture.
13. Centrifuge at 10,000 g for 1 minute to dry the column matrix.
14. 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!!.*

15. Incubate for 15 minutes at room temperature (15-25 °C).
16. 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.*

17. Add **500 µL WRNA-2 Buffer** (✓).

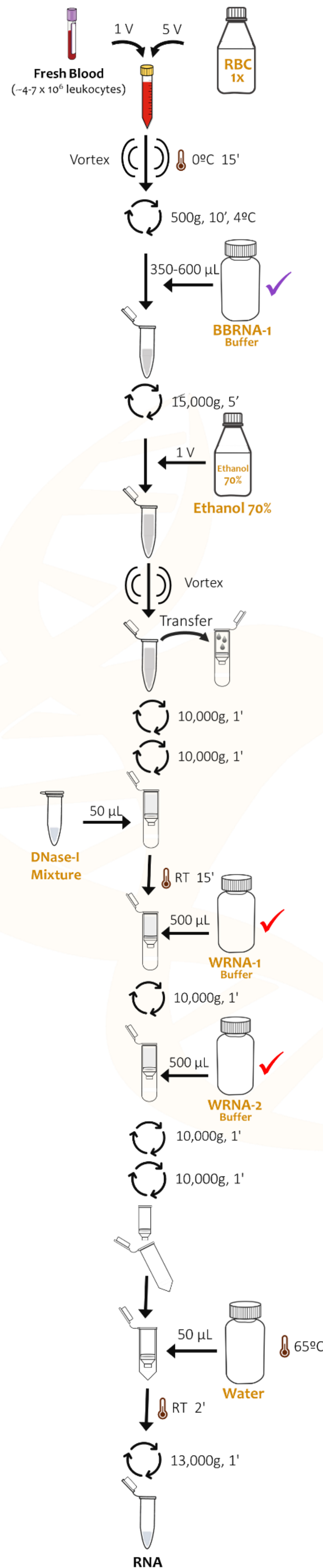
    ✓ *Check Ethanol has been added.*

18. Centrifuge at 10,000 g for 1 minute. Discard flow-through and place the High-Q™ RNA Spin Column back into the Collection Tube. Repeat step 17.
19. To dry silica matrix, centrifuge at 10,000g for 1 minute.
20. Place High-Q™ RNA spin column into a clean 1.5-mL microcentrifuge tube.
21. 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. Incubate for 1-2 minutes.
22. Centrifuge for 1 minute at 13,000 g. RNA isolated is in the eluate. Store RNA at -80°C.

© TIARIS Biosciences, 2023

TIARIS

FLOWCHART PROCEDURE



✓ β -mercaptoethanol has been added.

✓ Ethanol has been added.

For Research Use Only
© TIARIS Biosciences, 2023