High-Q™ Automated 16-Magnetic Tissue RNA Purification Kit

Ordering info

TBK0425-NP. 96 reactions

TBK0426-NP. 160 reactions

TBK0427-NP. 320 reactions

TBK0428-NP. 480 reactions

Description

High-Q™ Automated 16-Magnetic Tissue RNA Purification Kit is a new generation of nucleic acid purification system intended for automated purification. It is based on Tiaris-Mag™ magnetic beads, a homogenous silica-coated paramagnetic beads for purification of nucleic acids combined with an optimized lysis buffers that contains detergents, and reducing agents. Released nucleic acids are bound to the surface of Tiaris-Mag™ magnetic beads in the presence of a chaotropic salt. Nucleic acids bound to the beads are then efficiently washed and eluted using a magnetic separation device, removing contaminants.

Features

- Medium throughput.
- Quick and convenient RNA extraction from human and animal tissue (e.g. muscle, spleen, intestine, liver, heat, brain, rodent tail), insects, biopsy material.
- High yield and purity (A260/A280 ~2.0; A260/A230 ~2.0-2.2).

Applications

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.

Kit Components

Components	TBK0425-NP	TBK0426-NP	TBK0427-NP	TBK0428-NP
Tissue RNA-1 Buffer [a]	60 mL	100 mL	200 mL	3 x 100 mL
Tissue RNA-2 Buffer	10 mL	10 mL	20 mL	30 mL
DNase I (5 U/µL)	500 µL	815 µL	2 x 815 µL	2 x 1.25 mL
DNase-I Buffer 10x	25 mL	40 mL	70 mL	100 mL
WRNA-1 Buffer	20 mL[b]	36 mL[d]	72 mL[f]	100 mL[h]
WRNA-2 Buffer	32 mL[c]	3 x 20 mL[e]	3 x 40 mL[g]	4 x 40 mL[g]
Tiaris™Mag Beads	30 mL	50 mL	100 mL	2 x 75 mL
Water, nuclease free	25 mL	25 mL	50 mL	100 mL

Order Info Kit Components: *Tissue-RNA1 Buffer (TBB0581) | Tissue-RNA2 Buffer (TBB0582) | DNase-I (TBZ0320) | 10x DNase Buffer (TBB0319) | WRNA-1 Buffer (TBB0544) | WRNA-2 Buffer (TBB0545) | Tiaris™ Mag Beads (TBR0270) | Water, nuclease free (TBB0302).*

Before its use

[a] Add 10 µL β-mercaptoethanol per 1 mL Tissue RNA-1 Buffer.

[b] Add 30 mL isopropanol.

[c] Add 128 mL absolute ethanol.

[d] Add 54 mL isopropanol.

[e] Add 80 mL absolute ethanol each.

[f] Add 108 mL isopropanol.

[g] Add 160 mL absolute ethanol each.

[h] Add 150 mL isopropanol.

Storage

Store DNase at -20°C.

Store Tiaris™ Mag Beads at 4°C.

Store all other components at 25 °C.

Reagents required *(not provided)*

- Absolute ethanol (CAS 64-17-5).
- Isopropanol (CAS 67-63-0).
- Deep-well plates.
- 8 tip combs (TBM0035).

PROTOCOL

I. Preparation of processing plates for 8-tip combs robotic system

Each plate allows the isolation of 16 samples. Two plates can be run in parallel.

During solution storage precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37 °C and gently shaking before fill the plates.

	1	2	3	4	5	6	7	8	9	10	11	12
A	DNase-I Buffer (10x)	Isopropanol	WRNA-2 Buffer	WRNA-2 Buffer	Water, nuclease free	TIARIS-Mag™ Beads	DNase-I Buffer (10x)	Isopropanol	WRNA-2 Buffer	WRNA-2 Buffer	Water, nuclease free	TIARIS-Mag™ Beads
B												
C												
D												
E												
F												
G												
H												

- Columns 1 and 7: Add 200 µL DNase-I Buffer (10x)
- Columns 2 and 8: Add 300 µL Isopropanol (CAS 67-63-0)
- Columns 3, 4 and 9, 10: Add 800 µL WRNA-2 Buffer

  ¡ Check absolute ethanol has been added to WRNA-2 Buffer!
- Columns 5 and 11: Add 100 µL Water nuclease free
- Columns 6 and 12: Add 300 µL Tiaris-Mag™ Beads

  Shake Tiaris-Mag™ Beads bottle well or place it on a vortex shortly. Tiaris-Mag™ Beads must be distributed homogenously for a high well-to-well consistency. Therefore, before distributing the beads, make sure that they are completely resuspended.

II. Sample Preparation

1. Grind between 50-100 mg of fresh or frozen tissue sample by mechanical disruption. You could use liquid nitrogen and a mortar and a pestle or a commercially homogenizer equipment.
2. Add 600 µL Tissue RNA-1 Buffer and mix by vortex vigorously.

   ¡ Check Tissue RNA-1 Buffer includes β-mercaptoethanol !
3. Add 60 µL Tissue RNA-2 Buffer. Vortex briefly. Vortex vigorously for at less 30 seconds.
4. Incubate at 55°C, 5 minutes.
5. Centrifuge at 13,000 g for 5 minutes. Transfer the supernatant to the prefilled plate (III, step 4).

III. Automatized Nucleic Acid Purification from Tissue Sample

1. Check that there are not plates or combs in the equipment. Turn on and start instrument calibration.
2. Put the deep-well plate at room temperature.
3. Check that plate is properly oriented, that is, that A1 well is at left upper corner and add **5 µL of DNase-I** to wells in the columns 1 and 7.
4. **Add 400 µL of samples** to wells in the columns 2 and 8.
5. Plug 8-strip comb into the rack for tip insertion in the instrument (*see manual of instrument for details*).
6. Put 96-well plate into the instrument with A1 well at left upper corner.
7. Set up the instrument using the Program 1.

PROGRAM 1_STEPS	01	02	03
WELL/ HOLE	6	2	1
NAME	BEADS	BIND	DNASE
WAIT TIME*	0:00	0:00	3:00
MIX TIME*	0:30	5:00	10:00**
MAG TIME*	1:00	1:00	1:00
TEMPERATURE			37°C
VOLUME (µL)	300	700	200
MIXING METHOD	Fast	Slow	Slow
COLLECTION METHOD	Strong	Strong	Strong

* *Minutes : Seconds*
** *The Mix Time is customized, 1 minute mixing, 9 minutes paused*

8. Once the program has finished, take off the plate and add **500 µL WRNA1-Buffer** to wells in the columns 1 and 7.
   ¡ Check isopropanol has been added to WRNA-1 Buffer!
9. Put deep well plate into the instrument with A1 well at left upper corner.
10. Set up the instrument using the Program 2.

PROGRAM 2_STEPS	01	02	03	04	05
WELL/ HOLE	1	3	4	5	6
NAME	BIND	WASH I	WASH II	ELUTION	BEADS
WAIT TIME*	0:00	0:00	0:00	3:00	0:00
MIX TIME*	3:00	2:00	2:00	2:00	0:30
MAG TIME*	1:00	1:00	1:00	2:00	0:00
TEMPERATURE				70°C	
VOLUME (µL)	700	800	800	100	300
MIXING METHOD	Medium	Medium	Medium	Slow	Fast
COLLECTION METHOD	Strong	Strong	Strong	Strong	Normal

* *Minutes : Seconds*

11. Once the program has finished, recover eluted nucleic acid from each well on columns 5 and 11.

It is possible that a few beads might accidentally be transferred along with the final DNA sample, but this is unlikely to interfere with any subsequent applications. However, if you prefer, an additional separation step by centrifugation or magnetic separation can be carried out to remove the beads.

12. Store RNA at -80°C.

13. Remove the plugs and discard them and used plates according your local safety regulations.