TIANGEN®

TGuide Smart Blood/Cell/Tissue RNA Kit

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TGuide Smart Blood/Cell/Tissue RNA Kit

Cat. no. 4993551

Kit Contents

	Contents	4993551 (48 preps)
4993544	Buffer RL	30 ml
	Buffer RD	12 ml×2
	Blood/Cell/Tissue RNA Reagents	48
	Proteinase K	1 ml
	RNase-Free ddH_2O	15 ml
4992232	Buffer RDD	4 ml
	RNase-Free ddH_2O	1 ml
	RNase-Free DNase I (1500 U)	1 pcs
4993546	TGuide Smart Tip Comb	12 pcs

Note: 4992232 ,4993546 are shipped and packaged separately

Blood/Cell/Tissue RNA reagent composition

Well 1	Well 2	Well 3	Well 4	Well 5	Well 6
Buffer IC	MagAttract Suspension LSP2	None	Buffer RWP	None	Buffer RWP
450 µl	900 µl		700 µl		700 µl

Storage condition

All components of the kit can be stored in dry conditions at room temperature (15-30°C) for 12 months. RNase-free DNase I, RDD buffer and RNase-free ddH_2O(in tubes) can be stored at 2-8°C for 12 months.

Product

The kit adopts unique magnetic beads and a unique buffer system, to isolate and purify total RNA with high quality from blood/cell/tissue. The uniquely embedded magnetic beads have a strong affinity for nucleic acid under certain conditions. When the conditions are changed, the magnetic beads can release the adsorbed nucleic acid, to rapidly separate and purify the nucleic acid.

It can be used to perfectly fit with TGuide S16 Nucleic Acid Extractor. Through adsorption, transfer and release of magnetic beads by the special magnetic bar, magnetic beads and nucleic acid can be transferred, to improve the degree of automation. The whole process is safe and convenient, and the extracted RNA has high purity. If high throughput automated extraction is required, TIANGEN can deliver integration solutions.

RNA purified with this kit is suitable for downstream experiments including RT-PCR, Real Time RT-PCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, in vitro translation, RNase protection analysis and molecular cloning.

Features

- **Simple and fast:** Ultra-pure total RNA can be obtained by running TGuide S16 for 59 minutes .
- **Flexible flux:** It is perfect fit for TIANGEN's TGuide S16 automatic Nucleic acid extraction and purification instrument to extract 1-8 samples.
- **Safe and non-toxic:** No toxic reagents such as phenol/chloroform.
- **High purity:** The RNA obtained has high purity and can be directly used for chip detection, high-throughput sequencing and other experiments.

Notes

1. The product can be used with the TGuide S16 Nucleic Acid Extractor.
2. Pay attention to the optimal storage and pre-processing conditions of samples to avoid degradation of extracted RNA.
3. During pre-processing of fresh blood samples, the customer is required to prepare 10×Red cell lysis buffer H(TIANGEN, RT125) and RNase-Free Columns CS(TIANGENG, RK176).
4. Preparation of DNase I storage solution: Dissolve DNase I dry powder (1500 Unitz units) in 550 μl RNase-free ddH_2O, and mix them gently, and subpackage and store the solution at -30 ~ -15°C (up to 9 months). Take the solution out each time according to experimental requirements. Please note that the melted

DNase I storage solution should be stored at 2-8°C (up to 6 weeks). Do not freeze it again.

Preparation of DNase I working solution: For each reaction, take 10 μl DNase I storage solution, to which, add 70 μl Buffer RDD and mix them gently.

Operational steps

Before first use, add anhydrous ethanol into Buffer RD according to the volume as stated on the label of bottle.

I. Sample pre-processing

A. Tissue sample

1. Homogenate of tissue samples

   Note: The amount of tissue must be controlled within 20 mg, which may lead to a decrease in RNA yield and quality. For DNA-rich samples, such as the spleen, 5 mg is recommended.

   Take fresh or -80°C frozen plant tissue to fully ground with liquid nitrogen into powder. Weigh 5-20 mg to a 1.5 ml centrifuge tube containing 450 μl lysate RL and 20 μl Proteinase K, (or after adding proper amount of tissue into lysate, perform the electric homogenate), for vortex mixing immediately. Stand it at room temperature for 5 min, centrifuge at 12000 rpm for 5 min, and carefully absorb the supernatant.

2. Add the supernatant of above tissue (not more than 500 μl) to the deep hole plate of Buffer IC.

B. Cell samples

1. Processing methods of different cell samples:

   (1) Suspension cell: Estimate the number of cells collected (the collected number should not be more than $1×10^7$), to centrifugate at 300xg for 5min, and collect cells into a centrifuge tube and carefully absorb all supernatant from the culture medium.

   (2) Anchorage-dependent cell: Determine the number of cells and remove the medium. Wash cells with the PBS solution, and suck out the PBS solution, and add the PBS solution containing 0.10-0.25% trypsin to cells for processing them. When the cells are released from the wall of the vessel, add a medium containing serum to inactivate trypsin. Transfer the cell solution to an RNase-free centrifuge tube and centrifuge it at 300×g for 5 min. Collect cell precipitates and carefully absorb all supernatant.

Note: When collecting cells, it is important to remove all cell culture medium; otherwise, it will lead to incomplete pyrolysis, affect the binding of RNA and magnetic beads, resulting in the reduction of RNA production.

2. Add appropriate amount of buffer RL and 20 μl Proteinase K to the collected cell precipitate, and mix them vortically immediately, and stand the mixture at room temperature for 5 min.

   For the specific amount of lysate RL, please refer to the table below:

Number of precipitation cells	Buffer RL (μl)
<5×10^6	450
5×10^6-1×10^7	600

3. Add the treating fluid of above tissue (not more than 500 μl) to the deep hole plate of Buffer IC.

C. Fresh blood samples

Note: This kit is only suitable for extracting total RNA from fresh blood, instead of frozen blood.

1. Dilution of erythrocyte lysate: Select an appropriate volume of 10×Red cell lysis buffer H based on the volume of processed blood samples (Self-provided: TIANGEN, RT125) (For example, if the volume of blood sample to be process is 200 μl, then take 140 μl 10×Red cell lysis buffer H), and dilute the solution to 1×Red cell lysis buffer H with RNnase-free ddH_2O.
2. Add 5 times volume 1×Red cell lysis buffer H to 1 time volume of fresh whole blood (the customer needs to provide clean tubes).

   Note: a. For best mixing, the volume of the mixture of blood and 1×Red cell lysis buffer H should be lower than 3/4 of the tube volume. If the blood sample has high white blood cells, the volume of blood used can be reduced proportionally and the volume of Buffer RL used in step 7 should be also adjusted accordingly. b. The fresh whole blood only refers to that from mammals; This step is not suitable for the poultry because red cells contain nucleic acid, and whole blood lysis can be performed directly according to the initial blood amount required (i.e., step 7).
3. Incubate the mixture on ice for 10-15 min, and perform vortex mixing twice during the incubation process.

   Note: The solution will become translucent during the incubation, indicating red cells lysis. If necessary, the incubation time can be extended to 20 min.
4. Perform the centrifugation at 2,100 rpm (~400×g) for 10 min at 4°C, and remove

the supernatant completely.

Note: White blood cells may form pellets after the centrifugation, and make sure complete removal of the supernatant. The presence of trace red blood cells will make the pellets of white cells become red, which will disappear in the subsequent rinsing step.

5. Add 1×Red cell lysis buffer H to the leukocyte precipitate (the volume of adding 1×Red cell lysis buffer H is twice the amount of whole blood in step 1) and resuscitate cells.
6. Perform the centrifugation at 2,100 rpm (~400×g) for 10 min at 4°C, and remove the supernatant completely.
7. Add Buffer RL and 20 μl Proteinase K to the leukocyte precipitate (The amount of Buffer RL should be considered according to the table below), followed by vortex mixing or pipettor mixing.

   Note: If the blood is not the whole blood of a healthy person, the volume of Buffer RL should be determined based on the number of white cells in the blood. In this case, the cells should be completely lysed, without massive cell pellet.
8. (Optional) Transfer the solution to the filtration column CS (placed in the collecting tube) to centrifugate it at 12,000 rpm (~13,400×g) for 2 min. Discard the filtration column CS (TIANGEN, RK176) and collect the filtrate.

   Note: This step is recommended when clotting points or tablets exist, otherwise it will affect the subsequent binding and purification of nucleic acid and magnetic beads.
9. Add the above lysate (not more than 500 μl) to the deep hole plate of Buffer IC.

II. Operation steps of TGuide S16 Nucleic Acid Extractor

1. Pre-packaged single reagent

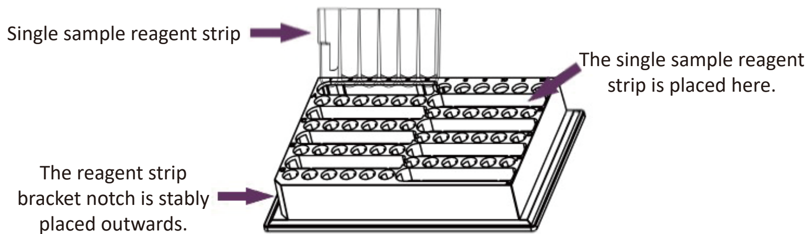
1.1 Take out a pre-packaged single sample reagent strip and mix it upside down several times to re-suspend the magnetic beads; Gently shake the reagent and magnetic beads to concentrate at the bottom of the orifice plate. Before use, remove sealing film carefully to avoid the orifice plate from vibrating and liquid spatter.

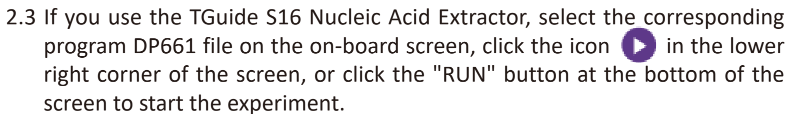
1.2 Add suitable volume (60-100 μl) of RNase-Free ddH_2O to the 5th well of single sample reagent strip.

2. Start the TGuide S16 Nucleic Acid Extractor

2.1 Add 450 μl supernatant obtained after processing the above sample to the 1st well of the single sample reagent strip and 10 μl pre-prepared DNase I storage solution and 70 μl Buffer RDD to the 3rd well. Place the single sample reagent strip on the reagent tank bracket of TGuide S16 Nucleic Acid Extractor.

2.2 Place the reagent tank bracket on the base of the 96-hole plate of the TGuide S16 Nucleic Acid Extractor. Insert the Tip Comb into the slot of the Tip Comb to ensure that they are well connected and firmed.





2.3 If you use the TGuide S16 Nucleic Acid Extractor, select the corresponding program DP661 file on the on-board screen, click the icon ▶ in the lower right corner of the screen, or click the "RUN" button at the bottom of the screen to start the experiment.

The extraction process of S16 provided for DP661 is shown in the following table:

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volume (µl)	Temp. (°C)	Segments	Every time(s)	Magneti-zation time(s)	Cycle	Magnet speed (mm/s)
1	1	Lysis	5	8	0	900	--	1	0	0	0	--
2	2	Collect beads	0.5	7	0	900	--	5	5	3	2	2
3	1	Bind	5	8	0	900	--	5	5	3	2	2
4	2	Wash1	5	7	0	900	--	5	5	0	2	2
5	3	DNase I	12	3	6	80	--	1	0	0	0	--
6	3	Pause	Add 700 µl Buffer RD into the 3rd well									
7	3	Wash 2	5	7	0	780	--	5	5	3	2	2
8	4	Wash 3	3	7	0	700	--	5	5	0	2	2
9	6	Wash 4	3	7	6	700	--	5	5	0	2	2
10	5	Elution	5	7	0	80	45	5	5	5	2	2
11	6	Discard	0.5	5	0	700	--	1	0	0	0	--

2.4 At the end of the automated extraction process, attract the RNA out of the 5th well of the single sample reagent strip and store it under appropriate conditions. If there are subsequent experiments, it can be stored at 4°C for no more than 4 hours.