TIANGEN®

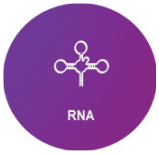
TGuide Smart Blood/Cell/ Tissue RNA Kit

For purification of RNA from Tissue, Cells, Blood, etc.

TECHNICAL MANUAL

Cat. no. 4993551

Note: To use the TGuide Smart Blood/Cell/Tissue RNA Kit, you must have the TGuide Smart Blood/Cell/Tissue RNA (program no. DP661 and non-stop program DP661-01) installed on the TGuide S16/S32 pro Nucleic Acid Extractor.



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This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetic

Table Contents

TGuide Smart Blood/Cell/Tissue RNA Kit

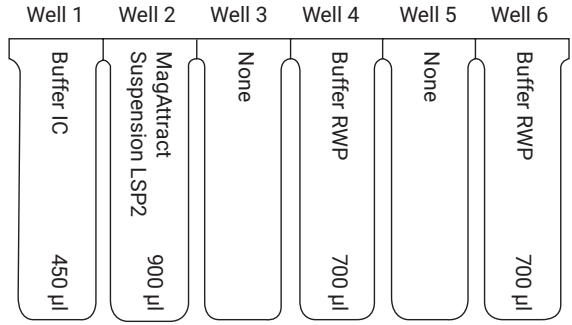
Cat. no. 4993551

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



Storage condition

All components of the kit 4993544 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 15 months.

Product

The kit adopts 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 absorbed nucleic acid to rapidly separate and purify it.

It can be used to perfectly fit with TGuide S16 Nucleic Acid Extractor. Through absorption, 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 integrated solutions.

RNA purified by 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 throughput:** It is a perfect fit for TIANGEN's TGuide S16 Nucleic Acid Extractor 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. DP661 program is suitable for all sample types. Pay attention to the pause step of DP661 (~30mins after running). Users need to manually add 700μl Buffer RD into the 3rd well before the program can resume. DP661-01 is a non-stop program that is suitable for nucleic acid-rich samples. Users don't need to add Buffer RD manually when DP661-01 is chosen.
3. Pay attention to the optimal storage and pre-processing conditions of samples to avoid degradation of extracted RNA.
4. During pre-processing of fresh blood samples, the customer is required to prepare 10×Red cell lysis buffer (not provided) and RNase-Free Columns CS (TIANGEN, 4991519, not provided).

Before the first use

1. Prepare DNase I stock solution : Dissolve the lyophilized DNase I (1500 units) in 550 μl of the RNase-free ddH_2O. **Do not remove the rubber top of the vial to prevent loss of DNase I powder. Use a syringe and a needle to inject the RNaseFree ddH_2O into the vial.** Mix gently by inverting. Do not vortex. Divide **the solution** into single-use aliquots, and store at -30~-15℃ for up to 9 months. Thawed aliquots can be stored at 2~8℃ for up to 6 weeks. Do not refreeze the aliquots after thawing.
2. Add absolute ethanol into Buffer RD according to the volume stated on the label of the bottle.

Operational steps

Sample pre-processing

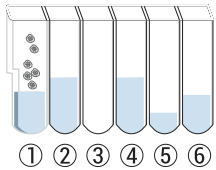
Tissue≤20mg → Homogenization

Cultured Cells≤1×10^7 → Harvest

Fresh Blood≤200μl → Red cell lysis

Add Buffer RL and Proteinase K, incubate 5min

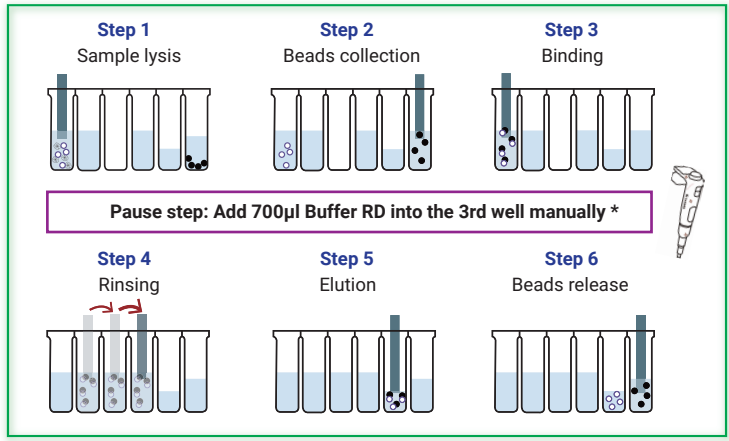
Cartridges preparation



1. Add 450μl above sample to well ①
2. Add 10μl DNase I and 70μl RDD to well ③
3. Add 60~100μl ddH_2O to well ⑤

Select program DP661 or DP661-01 on the touch screen

Automated extraction by S16



* DP661-01 is a non-stop program that is suitable for nucleic acid-rich samples. Users don't need to add Buffer RD manually when DP661-01 is chosen.

Pipette RNA from well ⑤

I. Sample pre-processing

A. Tissue sample

1. Tissue homogenate

   Note: The amount of tissue must be controlled within 20 mg. Larger input 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 tissue and fully grind with liquid nitrogen into powder. Weigh 5~20 mg to a 1.5 ml centrifuge tube containing 450 µl Buffer RL and 20 µl Proteinase K, (or after adding proper amount of tissue into lysis buffer, perform the electric homogenization), vortex mixing immediately at high speed for 30~45 sec. Let it stand at room temperature for 5 min, centrifuge at 12000 rpm for 5 min, and carefully take the supernatant.

2. Proceed to Part II, Step 1.

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) and centrifugate at 300xg for 5min. Then collect cells into a centrifuge tube and carefully remove all supernatant of the culture medium.

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

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

2. Add appropriate amount of buffer RL and 20 µl Proteinase K to the collected cell pellets, vortex 30~45 sec at high speed for homogenization, and let the mixture stand at room temperature for 5 min.

   For the volumn of Buffer RL, please refer to the table below:

Number of cells	Buffer RL (µl)
$<5\times10^6$	450
5×10^6~1×10^7	600

3. Proceed to Part II, Step 1.

Note. For cell pellet stored in Trizol or other ready-to-use reagent for the isolation of total RNA, equilibrate the freezing sample to RT. Vortex 30~60 sec at high speed for homogenization. Incubate the homogenate for 5min at RT. Proceed to Part II, Step 1 and add 20 µl Proteinase K to the 1st well of single sample cartridge.

C. Fresh blood samples

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

1. Dilution of Red cell lysis buffer: Take 10X Red cell lysis buffer for an example. Select an appropriate volume of 10×Red cell lysis buffer based on the volume of processed blood samples. For each 200 µl blood sample, 140 µl 10X Red cell lysis buffer in total must be diluted to 1XRed cell lysis buffer with RNase-free ddH_2O. Scale the volume of 10X Red cell lysis buffer up or down according to the volume of blood sample to be processed.
2. Add 5 times volume of 1×Red cell lysis buffer to 1 time volume of fresh whole blood (the customer needs to prepare clean tubes).

   Note: a. For best mixing, the volume of the blood and 1×Red cell lysis buffer should be lower than 3/4 of the tube volume. If the blood sample has large proportion of white blood cells, the volume of blood being 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 blood because poultry 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 are lysed. 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. 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 to the white blood cell pellets (the volume should be twice of the whole blood volumn in step 1) and resuspend 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 white blood cell pellets (The amount of Buffer RL should refer to the table below), followed by vortex mixing or pipette

mixing.

Note: If the blood sample is not collected from 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 cell pellet left.

Buffer RL(μl)	Healthy human whole blood (ml)	White blood cells
400	<0.5	2×10^6
600	0.5~1.5	2×10^6~1×10^7

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, 4991519) and collect the filtrate.

   Note: This step is recommended when clots are present which will affect the subsequent binding and purification of nucleic acid and magnetic beads.

9. Proceed to Part II, Step 1.

II. Operation steps of TGuide S16 Nucleic Acid Extractor

1. Prefilled single sample cartridge

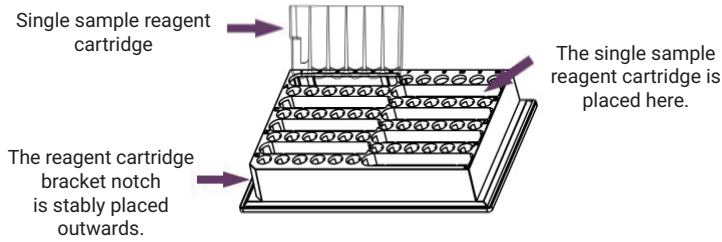
   1.1 Take out a prefilled single sample cartridge and invert it to re-suspend the magnetic beads; Gently shake to concentrate the reagent and magnetic beads to the bottom of the cartridge. Before use, remove sealing film carefully to avoid liquid spatter or spills.

   1.2 Add proper volume (60~100 μl) of RNase-Free ddH_2O to the 5th well of the cartridge.

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 cartridge. Add 10 μl pre-prepared DNase I stock solution and 70 μl Buffer RDD to the 3rd well. Place the cartridge on the reagent tank bracket of TGuide S16 Nucleic Acid Extractor.

   2.2 Place the reagent tank bracket on the plate base in the TGuide S16 Nucleic Acid Extractor. Insert the Tip Combs into the slots 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 or DP661-01 file on the touch screen, click the icon in the lower right corner of the screen and click the "RUN" button at the bottom of the screen to start the experiment.

2.4 At the pause step, add 700μl Buffer RD into the 3rd well manually and then click "Confirm".

Note: DP661-01 is a non-stop program that is suitable for nucleic acid-rich samples. Users don't need to add Buffer RD manually when DP661-01 is chosen.

2.5 At the end of the automated extraction process, take the RNA out of the 5th well of the single sample reagent cartridge and store it under appropriate conditions. If there are subsequent experiments, it can be stored at 4°C for no more than 4 hours. Single sample reagent cartridge and tip comb are for single use only.

Appendix

1. Program

1.1 The extraction process of S16 provided for DP661 is shown in the following table:This program is suitable for all sample types.

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Segments	Every time (sec)	Magnetization time (sec)	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	3	7	0	900	--	5	5	0	2	2
5	3	DNase I	12	3	0	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	--

1.2 The extraction process of S16 provided for non-stop DP661-01 is shown in the following table. DP661-01 is suitable for nucleic acid rich samples.

Step	Hole site	Step name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Segments	Every time (sec)	Magnetization time (sec)	Cycle	Magnet speed (mm/s)
1	2	Collect beads	0.5	7	0	900	--	5	5	3	2	2
2	1	Lysis	5	8	0	900	--	1	0	0	1	2.5
3	1	Bind	5	8	0	900	--	5	5	3	2	2
4	2	Wash 1	2	8	0	900	--	5	5	0	2	2.5
5	3	Dnase I	12	3	0	80	--	5	5	0	1	2.5
6	2	Wash 2	5	8	0	900	--	5	5	0	2	2
7	4	Wash 3	3	8	0	700	--	5	5	0	2	2
8	6	Wash 4	3	8	6	700	--	5	5	3	2	2
9	5	Elution	5	7	0	80	45	5	5	5	2	2
10	6	Discard	0.2	5	0	500	--	1	0	0	1	2.5

2. Related Products

Instrument and Accessories

Product name	Packing Size	Cat.No
TGuide S16 Nucleic Acid Extractor	1 set	OSE-S16-AM
TGuide Smart Magnetic Tip Comb	200 pieces/box	4968939
TGuide Single Sample Tank Bracket	5 pieces/box	4993270

TGuide Smart Reagent Kits

Product name	Packing Size	Cat.No
TGuide Smart Magnetic Plant DNA Kit	48 preps	4993548
TGuide Smart Soil /Stool DNA Kit	48 preps	4993549
TGuide Smart Magnetic Tissue DNA Kit	48 preps	4993547
TGuide Smart Magnetic Plant RNA Kit	48 preps	4993552
TGuide Smart DNA Purification Kit	48 preps	4993550
TGuide Smart Blood Genomic DNA Kit	48 preps	4993703
TGuide Smart Viral DNA/RNA Kit	48 preps	4993702
TGuide Smart Universal DNA Kit	48 preps	4993704