

TGuide Smart Blood/Cell/ Tissue RNA Kit

(Prefilled 96-Deepwell plate)

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

TECHNICAL MANUAL

Cat. no. GDP661-E

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 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

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TGuide Smart Blood/Cell/Tissue RNA Kit

Cat. no. GDP661-E

Kit Contents

	Contents	GDP661-E (96 preps)
GDP661H	Buffer RL	60 ml
	Buffer RD	48 ml
	Blood/Cell/Tissue RNA Reagents	6 plates
	Proteinase K	2×1 ml
	RNase-Free ddH ₂ O	15 ml
	TGuide Smart Tip Comb	12 pcs
GRT411	Buffer RDD	2×4 ml
	RNase-Free ddH ₂ O	2×1 ml
	RNase-Free DNase I (1500 U)	2×1 pcs

Note: GDP661H, GRT411 are shipped and packaged separately

Blood/Cell/Tissue RNA reagents composition

Column 1/7	Column 2/8	Column 3/9	Column 4/10	Column 5/11	Column 6/12
Buffer IC	MagAttract Suspension LSP2	None	Buffer RWP	None	Buffer RWP
450 µl	900 µl		700 µl		700 µl

Storage

All components of the kit GDP661-E can be stored in dry conditions at room temperature (15~30°C) for 12 months. RNase-Free DNase I, Buffer RDD and RNase-Free ddH₂O (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 1~16 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 Please be sure to read this precaution before using the kit.

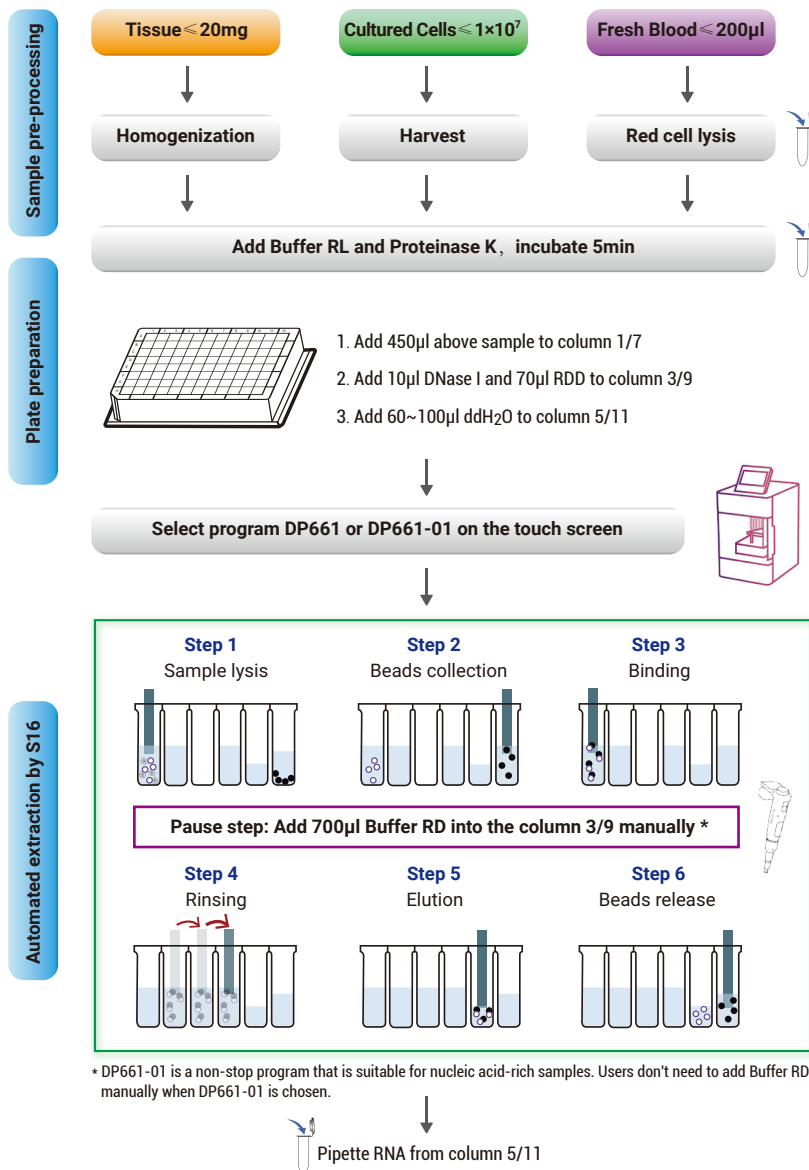
1. The product can be used with the TGuide S16 Nucleic Acid Extractor.
2. DP661 program is suitable for all sample types. Pay attention to the pause step of DP661 (~30mins after running). Users need to manually add 700µl Buffer RD into the 3rd and 9th columns before the program can resume. DP661-01 is 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. For sample stored in DNA and RNA stabilization solution, we recommend centrifuge to remove the storage buffer before sample pre-processing.
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, GRK176, not provided).

Before the first use

1. Prepare DNase I stock solution : Dissolve the lyophilized DNase I (1500 units) in 550 μ l of the RNase-Free ddH₂O. **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 RNase-Free ddH₂O into the vial.** Mix gently by inverting. Do not vortex. Divide the solution into single-use aliquots, and store at -30~-15°C for up to 9 months. Thawed aliquots can be stored at 2~8°C 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



* 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.

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 300xg 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)
$<5 \times 10^6$	450
$5 \times 10^6 \sim 1 \times 10^7$	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 μ l Proteinase K to the column 1/7 of 96-Deepwell plate.

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 10 \times Red cell lysis buffer for an example. Select an appropriate volume of 10 \times Red cell lysis buffer based on the volume of processed blood samples. For each 200 μ l blood sample, 140 μ l 10 \times Red cell lysis buffer in total must be diluted to 1 \times Red cell lysis buffer with RNase-free ddH₂O. Scale the volume of 10 \times Red cell lysis buffer up or down according to the volume of blood sample to be processed.

2. Add 5 times volume of 1 \times 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 \times 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 (\sim 400 \times g) for 10 min at 4 $^{\circ}$ 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 \times Red cell lysis buffer to the white blood cell pellets (the volume should be twice of the whole blood volume in step 1) and resuspend cells.
6. Perform the centrifugation at 2,100 rpm (\sim 400 \times g) for 10 min at 4 $^{\circ}$ C and remove the supernatant completely.
7. Add Buffer RL and 20 μ l 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	$2 \times 10^6 \sim 1 \times 10^7$

8. (Optional) Transfer the solution to the filtration column CS (placed in the collecting tube) to centrifugate it at 12,000 rpm ($\sim 13,400 \times g$) for 2 min. Discard the filtration column CS (TIANGEN, GRK176) 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 96-Deepwell plate

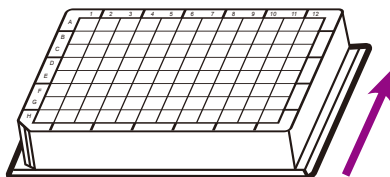
1.1 Take out a prefilled 96-Deepwell plate and invert it to re-suspend the magnetic beads; Gently shake to concentrate the reagent and magnetic beads to the bottom of the plate. Before use, remove sealing film carefully to avoid liquid spatter or spills.


1.2 Add proper volume (60~100 μl) of RNase-Free ddH₂O to the Column 5/11 of the plate.

2. Start the TGuide S16 Nucleic Acid Extractor

2.1 Add 450 μl supernatant obtained after processing the above sample to the Column 1/7 of the plate. Add 10 μl pre-prepared DNase I stock solution and 70 μl Buffer RDD to the Column 3/9.

2.2 Place the reagent plate on the 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 Column 3/9 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 Column 5/11 of the 96-Deepwell plate and store it under appropriate conditions. If there are subsequent experiments, it can be stored at 4°C for no more than 4 hours. 96-Deepwell plate 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	1/7	Lysis	5	8	0	900	--	1	0	0	0	--
2	2/8	Collect beads	0.5	7	0	900	--	5	5	3	2	2
3	1/7	Bind	5	8	0	900	--	5	5	3	2	2
4	2/8	Wash1	3	7	0	900	--	5	5	0	2	2
5	3/9	DNase I	12	3	0	80	--	1	0	0	0	--
6	3/9	Pause	Add 700 µl Buffer RD into the Column 3/9									
7	3/9	Wash 2	5	7	0	780	--	5	5	3	2	2
8	4/10	Wash 3	3	7	0	700	--	5	5	0	2	2
9	6/12	Wash 4	3	7	6	700	--	5	5	0	2	2
10	5/11	Elution	5	7	0	80	45	5	5	5	2	2
11	6/12	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	2/8	Collect beads	0.5	7	0	900	–	5	5	3	2	2
2	1/7	Lysis	5	8	0	900	–	1	0	0	1	2.5
3	1/7	Bind	5	8	0	900	–	5	5	3	2	2
4	2/8	Wash 1	2	8	0	900	–	5	5	0	2	2.5
5	3/9	Dnase I	12	3	0	80	–	5	5	0	1	2.5
6	2/8	Wash 2	5	8	0	900	–	5	5	0	2	2
7	4/10	Wash 3	3	8	0	700	–	5	5	0	2	2
8	6/12	Wash 4	3	8	6	700	–	5	5	3	2	2
9	5/11	Elution	5	7	0	80	45	5	5	5	2	2
10	6/12	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
TGuide Smart Magnetic Tip Comb	200 pieces/box	OSE-TGA-S03
TGuide Single Sample Tank Bracket	5 pieces/box	OSE-TGA-S32

TGuide Smart Reagent Kits

Product name	Packing Size	Cat.No
TGuide Smart Magnetic Plant DNA Kit	48 preps	GDP607-DE
TGuide Smart Soil /Stool DNA Kit	48 preps	GDP612-DE
TGuide Smart Magnetic Tissue DNA Kit	48 preps	GDP602-DE
TGuide Smart Magnetic Plant RNA Kit	48 preps	GDP662-DE
TGuide Smart DNA Purification Kit	48 preps	GDP642-DE
TGuide Smart Blood Genomic DNA Kit	48 preps	GDP601-DE
TGuide Smart Viral DNA/RNA Kit	48 preps	GDP604-DE
TGuide Smart Universal DNA Kit	48 preps	GDP605-DE
TGuide Smart Magnetic Tissue/Cell DNA Kit	96 preps	GDP602-E
TGuide Smart Blood/Cell Tissue RNA Kit	96 preps	GDP661-E
TGuide Smart Universal DNA Kit	96 preps	GDP605-E