TGuide S96 Blood/Cell/Tissue RNA Kit

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TGuide S96 Blood/Cell/Tissue RNA Kit

Cat.no. GDP861

Kit Contents

Contents	GDP861 (96 preps)
Buffer RL	60 ml
Buffer IC	1 plate (96×400 µl/well)
Buffer RDP	1 plate (96×900 µl/well)
Buffer RWP	1 plate (96×700 µl/well)
MagAttract Suspension WSP	1 plate (96×740 µl/well)
RNase-Free ddH_2O	1 plate (96×100 µl/well)
Buffer RD	48 ml
Proteinase K	2 × 1 ml
RNase-Free DNase I	1 tube
Buffer RDD	4 ml
RNase-Free ddH_2O	1 ml
KF Deep Well 96 Plate	1 plate
KF 96-Tip Comb	1 set
Handbook	1

Storage

This kit can be stored at room temperature (15-30°C) under dry condition for 12 months. RNase-Free DNase I, Buffer RDD and RNase-Free ddH_2O (tube) should be stored at 2-8°C for 12 months.

Introduction

The kit adopts magnetic beads with unique separation function and a unique buffer system to separate and purify high-quality total RNA from various tissues, cells and blood. The unique embedded magnetic beads have strong affinity for nucleic acid under certain conditions, and when the conditions change, the magnetic beads will release adsorbed nucleic acid, thus achieving the purpose of fast separation and purification of nucleic acid.

The product can be perfectly matched with TGuide S96 extractor. Magnetic beads are adsorbed, transferred and released by special magnetic rods, thus realizing the transfer of magnetic beads and nucleic acids. The whole experimental process is safe and convenient, and the extracted total RNA has high purity and is free from contamination of genomes, proteins and other impurities.

RNA purified by the kit is suitable for various downstream experiments such as RT-PCR, RT-qPCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, *in vitro* translation, RNase protection analysis, molecular cloning, etc.

Features

- **Easy and fast:** RNA with higher purity can be easily obtained in a short time by direct automatic extraction after sample treatment.
- **High throughput:** The kit can perfectly adapted with TGuide S96 Automated Nucleic Acid Extractor to performs high throughput extraction experiments.
- **Safe and nontoxic:** No toxic reagents such as phenol/chloroform are needed.
- **High purity:** The obtained RNA has high purity and can be directly used in chip detection, high-throughput sequencing and other experiments.

Notes <u>Please read these notes before using this kit</u>

1. This product is suitable for TGuide S96 Automated Nucleic Acid Extractor.
2. Pay attention to the storage and treatment conditions of samples to avoid degradation of extracted RNA.
3. For the treatment of fresh blood samples, the 10×Red cell Lysis Buffer H and RNase-Free Columns CS set should be self-provided.

4. Preparation of DNase I storage solution: Dissolve DNase I dry powder (1500 units) in 550 µl RNase-Free ddH_2O, mix gently and evenly, store at -30~-15°C after subpackaging (It can be stored for 9 months), and take out appropriate amount according to the experimental requirements each time. Please pay attention to store the thawed DNase I storage solution at 2-8°C for 6 weeks, and do not freeze it again. Preparation of DNase I working solution: Take 5 µl of DNase I storage solution for each reaction, add 70 µl Buffer RDD, and mix gently.

Protocol

Please add 96-100% ethanol to Buffer RD before using for the first time according to the label on the bottle.

I. Sample treatment

A. Tissue sample

1. Tissue sample homogenate

   Note: The amount of tissue should not exceed 20 mg, otherwise the yield and quality of RNA may decrease. For DNA-rich samples, such as spleen, 5 mg is recommended.

   Take fresh or frozen tissue at -80°C, grind with liquid nitrogen into powder. Weigh 5-20 mg into a 1.5 ml centrifuge tube filled with 450 µl of Buffer RL and 20 µl of Proteinase K (or add appropriate amount of tissue into Buffer RL, and homogenize electrically). Vortex and mix immediately, let it stand at room temperature for 5 min, centrifuge at 12,000 rpm for 5 min, and carefully remove the supernatant.

2. Take no more than 500 µl of the above supernatant and add it into the 96-deep-well plate of Buffer IC

B. Cell sample

1. Different cell sample processing methods:

   (1) Suspended cells: Estimate the number of collected cells (the number of collected cells should not exceed 1×10^7), centrifuge at 300×g for 5 min, collect the cells into a centrifuge tube, and carefully remove all the supernatant of the culture medium.

   (2) Adherent cells: Determine the number of cells, absorb the culture medium, wash the cells with PBS solution, absorb the PBS solution, add PBS solution containing 0.10-0.25% trypsin to the cells to treat the cells. When the cells are separated from the container wall, add the culture medium containing serum to inactivate trypsin, transfer

the cell solution to a RNase-Free centrifuge tube, centrifuge at 300×g for 5 min, collect the cell precipitate, and carefully remove all supernatant.

Note: When collecting cells, the cell culture solution must be removed completely, otherwise it will lead to incomplete lysis, affect the binding of RNA and magnetic beads, and reduce the yield of RNA.

2. Add appropriate amount of Buffer RL and 20 µl Proteinase K to the collected cell precipitate, vortex and mix immediately, and let it stand at room temperature for 5 min.

Please refer to the following table for the specific dosage of Buffer RL

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

3. Take no more than 500 µl of the above treated sample solution and add it into the 96-deep-well plate of Buffer IC.

C. Fresh blood sample

Note: This kit is only suitable for extracting total RNA from fresh blood, not frozen blood.

1. Dilution of 10×Red cell Lysis Buffer H: Choose an appropriate volume of 10×Red cell Lysis Buffer H (self-provided) according to the volume of the treated blood sample (for example, take 140 µl of 10×Red cell Lysis Buffer H if the volume of the blood sample to be treated is 200 µl), and dilute to 1×Red cell Lysis Buffer H with RNase-Free ddH_2O.
2. Add 5 volumes of 1× Red cell Lysis Buffer H to 1 volume of fresh whole blood (proper tubes should be provided).

Note: a. In order to obtain the best mixing effect, the volume of the mixed solution of blood and 1× Red cell Lysis Buffer H should not exceed 3/4 of the tube volume. If the white blood cell content in blood is high, the volume of blood used can be reduced proportionally, and the volume of Buffer RL used in step 7 should be adjusted accordingly. b. Fresh whole blood here refers only to mammals; Since the 10×Red cell Lysis Buffer H of poultry contain nucleic acid, this step is not required, and the initial amount of blood can be determined according to the requirements to directly perform whole blood lysis (i.e. step 7).

3. Incubate on ice for 10-15 min, and mix evenly for 2 times by vortex during incubation.

Note: During incubation, the solution will become translucent, indicating lysis of red blood cells. If necessary, the incubation time can

be extended to 20 min.

4. Centrifuge at 2,100 rpm (~400×g) for 10 min at 4°C to completely remove the supernatant.

   Note: White blood cells may form globules after centrifugation, please ensure complete removal of supernatant. The presence of trace amount of red blood cells will make white blood cell globules appear red, and this phenomenon will disappear in the subsequent washing step.

5. Add 1× Red cell Lysis Buffer H (the volume of 1× Red cell Lysis Buffer H added is 2 times of the total blood in the step 1) to leukocyte precipitation to resuspend cells.
6. Centrifuge at 4°C, 2,100 rpm (~400×g) for 10 min to completely remove the supernatant.
7. Add Buffer RL and 20 µl Proteinase K to leukocyte precipitation. Use the following table for Buffer RL, vortex or mix evenly with a pipette.

   Note: If the blood is not the whole blood of a healthy person, the volume of Buffer RL needed should be determined according to the number of white blood cells in the blood. At this time, the cells should be completely lysed and the massive cell precipitation will disappear.

Buffer RL (µl)	Healthy human	No. of white blood cells
400	Up to 0.5	Up to 2×10^6
600	0.5-1.5	2×10^6 to 1×10^7

8. **(Optional)** Transfer the solution to the RNase-Free Columns CS (the Column CS is placed in the collection tube), centrifuge at 12,000 rpm (~ 13,400×g) for 2 min, discard the Column CS, and collect the filtrate.

   Note: If there are coagulation spots or tablets, this step is recommended, otherwise it will affect the binding and purification of subsequent nucleic acids and magnetic beads.

9. Add no more than 500 µl of the aboved lysate into the 96-deep-well plate of Buffer IC.

II. Operation steps of TGuide S96

A. Preparation

Take out the vacuum package prepackaged 96-deep well plate from the kit, mix it upside down for several times to resuspend the magnetic

beads. Remove the vacuum package, gently shake the 96-deep-well plate to concentrate the reagent and magnetic beads to the bottom of the 96-deep-well plate (or centrifuge at 500 rpm for 1 min by the plate centrifuge). Carefully tear off the aluminum foil sealing film before use to avoid vibration of the 96-deep-well plate and prevent liquid spillage.

B.Reagent and Plate Distribution

Plate position	E	F	G	H
Reagent	Blank	DNase I working solution ★ 75 µl	Buffer WSP 740 µl Tip Comb	Blank
Plate position	**A**	**B**	**C**	**D**
Reagent	Buffer IC 400 µl Sample ▲ 500 µl	Buffer RDP 900 µl	Buffer RWP 700 µl	RNase-Free ddH_2O 100 µl

Note: ★ After washing with Buffer RDP, the instrument will set a pause step and the magnetic beads will be dried for 5 min. At the same time, add 75 µl DNase I working solution (5 µl DNase I storage solution +70 µl Buffer RDD) to each well of the empty 96-deep-well plate. Place the plate in the F plate position and continue slapping and mixing for 15 min. Set the pause step again to add 700 µl of Buffer RD to DNase I working solution of F plate (please check whether ethanol has been added first) and continue to run the program.

Note: ▲ Add no more than 500 µl of the treated samples to Buffer IC before loading to the machine, and place the 96-deep-well plate with the added samples and Buffer IC to the A plate position.

C. TGuide S96 automatic running program

1. Add the aboved treated samples to the 96-deep-well plate of Buffer IC. Put the KF 96-Tip Comb in the 96-deep-well plate of Magattact Suspension WSP. Lay out the machine according to the plate position distribution in step B.
2. Run the TGuide S96 program

The experimental workflow is shown in the following table:

Step	Plate Position	Mixing volume (μl)	Mixing speed	Mixing time (min)	Precipitation time (sec)	Adsorption times	Adsorption speed (mm/s)	Heating plate	Heating temperature (°C)	Suspension time (min)	Automatic pause	Capture mode
Capture Tip Comb	G	—	—	—	—	—	—	—	—	—	—	Capture
Collect Beads	G	740	Medium	0.5	2	1	1	—	—	—	—	—
Binding	A	900	Medium slow	10	2	1	1	—	—	—	—	—
Wash-I	B	900	Medium slow	3	2	1	1	—	—	5	Yes	—
DNase I	F	75	Medium	15	2	1	—	—	—	—	Yes	—
Wash-II	F	775	Medium	3	2	1	1	—	—	—	—	—
Wash-III	G	740	Medium	3	2	1	1	—	—	—	—	—
Wash-IV	C	700	Medium	3	2	1	1	—	—	5	—	—
Elution	D	100	Medium	8	4	2	1	D	60	—	—	—
Finish	G	—	—	—	—	—	—	—	—	—	—	Release

3. After the TGuide S96 automatic nucleic acid extraction and purification program is completed, transfer the DNA in the 96-deep-well plate to new tubes and store at -80°C.