TIANGEN®

TGuide Smart Universal RNA Kit

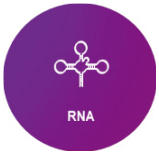
(Prefilled single sample cartridge)

For RNA extraction from a wide range of samples. Cultured cells, blood, animal tissues (performance will be better in high-fat and more viscous samples)

TECHNICAL MANUAL

Cat. no. GDP671-DE

Note: To use the TGuide Smart Universal RNA Kit, you must have the TGuide Smart Universal RNA (program no. DP671) 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 Universal RNA Kit

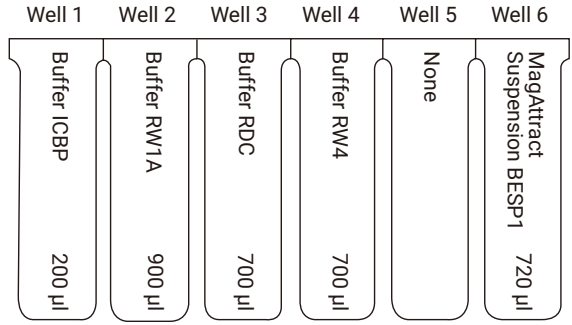
Cat. no. GDP671-DE

Kit Contents

Contents		GDP671-DE (48 preps)
GDP671-DH	RNAstore Reagent	6 ml
	Buffer TSA	35 ml
	Proteinase K	1 ml
	Universal RNA Reagents	48 pcs
	RNase-Free ddH_2O	15 ml
GRT431	RNase-Free DNase I (1500 U)	1 pcs
	RNase-Free ddH_2O	1 ml
OSE-TGA-S36	TGuide Smart Tip Comb	12 pcs

Note: GDP671-DH, GRT431 and OSE-TGA-S36 are shipped and stored separately.

Universal RNA Reagents Composition



Storage

RNase-Free DNase I, RNase-Free ddH_2O is placed at 2-8℃ and can be stored for 15 months. The other components of the kit can be stored for up to 12 months at room temperature (15-30°C) under dry conditions.

Introduction

The kit adopts magnetic beads and a unique buffer system, to isolate and purify total RNA with high quality from 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 automated 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: Automated extraction allows for easy access to higher purity RNA in a short time.

Safe and non-toxic: No toxic reagents such as phenol/chloroform.

High purity: The RNA obtained is of high purity and can be directly used for microarray detection, high-throughput sequencing and other experiments.

Notes **Please be sure to read this precaution before using the kit.**

1. This product is suitable for TGuide S16/S32/S32 Pro/S96 Dex fully automated nucleic acid extractor.
2. Wear a mask during the experiment and change new gloves frequently to avoid RNase contamination.
3. Avoid cross-contamination by using RNase-free plastics and tips.
4. Pay attention to the optimal storage and pretreatment conditions of the samples to avoid causing degradation of the extracted RNA.

Recommended dosage for different types of samples

Sample type		Optimal extraction amount
Tissue	Spleen	5 mg
	Liver	10 mg
	Kidney	15 mg
	Intestine	15 mg
	Gland	15 mg
	Heart	25 mg
	Lung	25 mg
	Brain	25 mg
	Fat	50 mg
	Skin	100 mg
	Muscle	100 mg
	Tail	0.3 cm for rats, 0.6 cm for mice
Cell	Adherent or suspension cells	Less than 10^7 cells
Insects	Mollusc	25 mg
	Crustacea	50 mg
Fungus	Yeast	Not more than 5×10^7
	Mould	25 mg
	Mushrooms	75 mg

Preparation of solutions for use with DNase I

Dissolve DNase I dry powder (1500 U) in 1 ml RNase-Free ddH_2O, mix gently and follow the protocol to add 10 μl to each sample for freshly use.

Attention: If long-term storage needed, dissolve DNase I dry powder (1500 U) in 550 μl RNase-Free ddH_2O to make stock solution, mix gently, dispense and store at -30~-15°C (can be stored for 9 months). Add 5 μl stock solution to each sample. Store the DNase I stock solution after thawing from -30~-15°C at 2-8°C (can be stored for 6 weeks) and do not freeze it again.

Protocol

1. Preparation of Universal RNA Reagents

1.1Take 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. Sample pre-treatment

2.1 Tissue sample

1) Grinding by liquid nitrogen. Take 5-100 mg tissue and quickly grind it to powder in liquid nitrogen, add 100 μl RNA store Reagent, 600 μl Buffer TSA and 20 μl Proteinase K. Immediately vortex and mix, let it stand at room temperature for 5 min.

2) Grinding by Homogenizer. Take 5-100 mg of tissue, add 100 μl of RNAstore Reagent and grinding beads (not provide, TIANGEN, Cat.no OSE-TH-B03). Homogenize with TGrinder H24R (not provide, TIANGEN, Cat.no OSE-TH-02) and homogenize with a program of oscillate 6.5 M/S for 30 sec, 1 cycle at -10°C. Add 600 μl Buffer TSA and 20 μl Proteinase K, vortex and mix, let it stand at room temperature for 5 min.

3) Centrifuge at 12,000 rpm (~13,400×g) for 5 min and carefully take 600 μl supernatant for subsequent experiments.

Note: Pre-treatment protocol for Trizol-preserved tissue samples

a. Using a pipette remove and discard all of the original Buffer ICBP from well 1 and refill with 250 μl of isopropanol.

b. After homogenization according to "2.1-2)", centrifuge at 8,000 rpm for 2 min, take 600 μl of supernatant and add it to well 1 (no need to add RNAstore preservation solution, Buffer TSA and Proteinase K), then continue to the subsequent process of step 3.1.

2.2 Cell sample

1) Collection of suspended cells: estimate the number of cells (please do not collect more than 1×10^7 cells), centrifuge the cells at 300×g for 5 min, collect the cells into centrifuge tubes, and carefully take all the medium supernatant.

2) Collection of adherent cells (trypsin treatment): estimate the number of cells (please do not collect more than 1×10^7 cells) and discard the medium. Wash the cells with PBS solution, discard the PBS solution, and add PBS solution containing 0.10-0.25% trypsin to detach the cell from the wall. Add medium containing serum to inactivate the trypsin then transfer the cell solution to

RNase-Free centrifugation tubes. Centrifuge at 300×g for 5 min, carefully discard all supernatant and collect the cell pellets.

3) Add 100 µl of RNAstore Reagent, 600 µl of Buffer TSA and 20 µl of Proteinase K to the cell pellets, vortex immediately, let it stand at room temperature for 5 min, and carefully take 600 µl of supernatant for subsequent experiments. Proceed to Step 3.1.

Note 1:

a. Be sure to remove the cell culture when collecting the cells, otherwise it will lead to incomplete lysis and will affect the binding of RNA to the magnetic beads, which resulting in a lower yield of RNA.

b. Leukocytes isolated from fresh whole blood can also refer to the cell extraction protocol, and the preservation of leukocytes can refer to the RNAstore Reagent (not provide, TIANGEN, Cat.no GDP408) process.

Note 2: Pre-treatment protocol for Trizol-preserved cell samples

a. Using a pipette, remove and discard all of the original Buffer ICBP from well 1 and refill with 250 µl of isopropanol.

b. The cell sample preserved in Trizol was vortexed and mixed and 600 µl of supernatant was added to well 1 (no need to add RNAstore Preservation Solution, Buffer TSA and Proteinase K), and Proceed to Step 3.1.

2.3 Blood sample

1) Rapid protocol for direct lysis of fresh anticoagulated blood.

Take 150 µl of fresh anticoagulant blood, add 450 µl of Buffer TSA and 20 µl of Proteinase K, immediately vortex and mix, and let it stand at room temperature for 5 min, carefully aspirate 600 µl supernatant for subsequent experiments.

2) Fresh anticoagulant pre-treatment program

a. Take no more than 1 ml of fresh anticoagulated blood solution and add RNALock reagent (not provide, TIANGEN, Cat.no GDP440-02) at a ratio of 1:5, e.g., take 300 µl of fresh anticoagulated whole blood and add 1.5 ml of RNALock reagent.

Note: Make sure RNALock reagent is stored at room temperature before use.

b. Immediately cap the tube and mix 8-10 times upside down.

Note: If storage is required, please refer to the storage conditions in the instructions for RNALock reagent (not provide, TIANGEN, Cat.no GDP440-02).

c. Centrifuge at 6,500 rpm (~4,000×g) for 10 min, discard the supernatant with a pipette, and take the precipitate for the follow.

Note: If clumping or insufficient cleavage is evident after centrifugation, repeat steps a-c.

d. Add 1 ml RNase-Free ddH_2O (not provide) to the precipitate and pipette to completely dissolve the precipitate.

e. Centrifuge at 6,500 rpm (~4,000×g) for 10 min and discard the supernatant by pipetting.

f. Slowly add 150 µl of Suspension RSB (not provide, TIANGEN, as a component of RNALock, Cat.no GDP440) and pipette repeatedly to dissolve the precipitate.

g. Add 450 µl of Buffer TSA and 20 µl of Proteinase K, immediately vortex and mix, let stand at room temperature for 5 min, and carefully aspirate 600 µl of supernatant for subsequent experiments.

3) Blood storage in RNAlock or PAXgene tube

a. To purify blood samples preserved in RNALock and PAXgene tube, first bring the samples to bench or a 37°C water bath to balance them to room temperature. Take appropriate volume of sample and centrifuge at 6,500 rpm (~4,000×g) for 10 min (The maximum extraction capacity is 1 ml whole blood for each prep). Pipette off the supernatant and take the precipitate for the following operation.

b. Add 1 ml of RNase-Free ddH_2O (not provide) to the precipitate and pipette to completely dissolve the precipitate.

c. Centrifuge at 6,500 rpm (~4,000×g) for 10 min and pipette off the supernatant.

d. Slowly add 150 µl of Buffer RSB (not provide, TIANGEN, as a component of RNALock, Cat.no GDP440) and pipette to dissolve the precipitate completely.

Note: 150 µl Buffer RSB can be replaced by 150 µl buffer PBS if using PAXgene tube.

e. Add 450 µl of Buffer TSA and 20 µl of Proteinase K, immediately vortex and mix, let stand at room temperature for 5 min, and carefully aspirate 600 µl of supernatant for subsequent experiments. Proceed to Step 3.1.

4) Trizol-preserved whole blood.

a. Using a pipette, remove and discard all of the original Buffer ICBP from well 1 and refill with 250 µl of isopropanol.

b. The whole blood sample preserved in Trizol was thoroughly vortex and 600 µl of supernatant was added to well 1 (no need to add RNAstore Preservation Solution, Buffer TSA and Proteinase K), and Proceed to Step 3.1.

2.4 Bacteria, fungi, environment, deep food processing category

a. Sample pre-treatment

1) Bacterial cultures

Centrifuge at 6,500 rpm (~4,000×g) at 4°C for 2 min to collect bacteria (the maximum amount of bacteria collected should not exceed 1×10^9), carefully remove all culture medium supernatant, and all subsequent steps are performed at room temperature .

Note: Incomplete removal of the medium will inhibit the cell wall digestion process.

2) Environmental samples

Silt and sediment type samples: Take 50-100 mg of silt and sediment type samples and add them to a centrifuge tube.

Water samples: Take a certain volume of water and filter it through a membrane, then cut up the membrane sample and put it into a centrifuge tube (or, depending on the sample, centrifuge it at high speed and add 50-100 mg of precipitate to the centrifuge tube for the next step).

3) Food samples

Yogurt samples: take 1-2 ml of sample and centrifuge at 4°C, 6,500 rpm (~4,000×g) for 2 min to collect the precipitate.

Soy sauce samples: take 10-50 ml of sample and centrifuge at 4°C, 6,500 rpm (~4,000×g) for 2 min to collect the precipitate.

Wine fermentation samples: Take 1-5 ml samples of fermentation intermediates and centrifuge for 2 min at 4°C, 6,500 rpm (~4,000×g) to collect the precipitate. Solid samples can be extracted by taking about 100-200 mg samples.

Note 1: Since the microbial content of deep-processed samples varies greatly at different stages of processing, a suitable sample volume can be taken according to the actual sample conditions.

Note 2: Samples with more impurities can be washed once, centrifuged to collect the precipitate, resuspended by adding 1 ml of RNase-Free ddH_2O, centrifuged at 6,500 rpm (~4,000×g) for 2 min to collect the precipitate and continue with subsequent experiments.

4) Yeast

Take 1-2 ml of yeast cells (up to 5×10^7 cells), centrifuge at 12,000 rpm (~13,400×g) for 1 min, and aspirate the supernatant as much as possible (when there is a large amount of bacterial fluid, the bacterial precipitate can be collected by several centrifugations into a centrifuge tube).

5) Mould

Mould cultured in liquid medium were collected by centrifugation at 12,000 rpm (~13,400×g) for 1 min, and 30-50 mg samples were taken for the next step.

When culturing with solid medium, scrape mycelium from the surface and take 30-50 mg of mycobacteria for the next step in the experiment.

Note: Mycobacterial samples can also be ground in liquid nitrogen, omitting the enzyme digestion of steps b and c, and proceeding directly to step d Add lysate for subsequent steps.

b. Thoroughly resuspend the bacteria with 400 µl of Buffer LY (not provided, TIANGEN, Cat.no GRT401-11), add 50 µl of Lysozyme A (not provided, 50 mg/ml, TIANGEN, Cat.no GRT401-11) and 2 µl of Lyticase A (not provided, 10 U/µl, TIANGEN, Cat.no GRT410-12), incubate at 37°C for 15 min.

Note: If only focusing on bacteria, you can add only Lysozyme A for digestion; If focusing on fungi only, you can add only Lyticase A for digestion.

c. Centrifuge at 6,500 rpm (~4,000×g) for 2 min and discard the supernatant.

d. Add 650 µl of Buffer TSA and 20 µl of Proteinase K. Suspend the precipitate and transfer it to a homogenized grinding tube (not provide, TIANGEN, Cat. no OSE-TH-B06), and mix at 1,200 rpm for 15 min. It can also be mixed (temperature -10°C, oscillation at 6 M/S for 30 sec, 1 cycle) using a TGrinder H24R Tissue Homogenizer (customer supplied, TIANGEN, Cat. no OSE-TH-02).

e. After homogenization, centrifuge at 12,000 rpm (~13,400×g) for 2 min, and carefully aspirate 600 µl of supernatant for subsequent tests. Proceed to Step 3.1.

2.5 Stool samples

a. Weigh 50-100 mg of fecal samples, use 400 µl of buffer LY (not provided, TIANGEN, Cat.no GRT401-11) to mix thoroughly. The sample should be thoroughly resuspended and dispersed as much as possible without obvious lumps.

Add 50 µl of lysozyme A (not provided, 50 mg/ml, TIANGEN, Cat.no GRT401-11) and 2 µl of lyticase A (not provided, 10 U/µl, TIANGEN, Cat.no GRT410-12) and incubate at 37°C for 15 min.

Note1: If only focusing on bacteria, you can add only Lysozyme A for digestion; If focusing on fungi only, you can add only Lyticase A for digestion.

Note 2: Samples with more impurities can be washed once, resuspended by adding 1 ml of RNase-Free ddH_2O, centrifuged at 6,500 rpm (~4,000×g) for 2 min to collect the precipitate, followed by enzymatic digestion experiments.

b. Centrifuge at 6,500 rpm (~4,000×g) for 2 min and discard the supernatant.

c. Add 650 µl of Buffer TSA and 20 µl of Proteinase K. Suspend the precipitate and transfer it to a homogenization tube (not provide, TIANGEN, Cat.no OSE-TH-B06), and shake and mix at 1,200 rpm for 15 min. It can also be mixed (temperature -10°C, oscillation at 6 M/S for 30 sec, 1 cycle) using a TGrinder H24R Tissue Homogenizer (customer supplied, TIANGEN, Cat.no OSE-TH-02).

d. After homogenization, centrifuge at 12,000 rpm (~13,400×g) for 2 min, and carefully aspirate 600 µl of supernatant for the subsequent experiments. Proceed to Step 3.1.

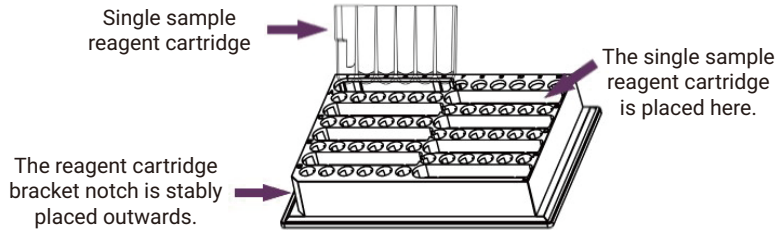
3. TGuide S16/S32/S32 Pro/S96 Dex Automatic Nucleic Acid Extractor

3.1 Add 600 µl supernatant after above treatments to the 1st well of the single sample cartridge and 10 µl of DNase I to well 3.

Note: To avoid affecting the activity of DNase I, please add it freshly to well 3 before running.

3.2 Place the cartridge on the reagent tank bracket of TGuide S16/S32/S32 Pro/S96 Dex Nucleic Acid Extractor.

3.3 Place the reagent tank bracket on the plate base in the TGuide S16/S32/S32 Pro/S96 Dex Fully Automated Nucleic Acid Extractor and insert the tip comb into the slot and make sure they are well connected and firmed.



3.4 Select the corresponding experiment program of TGuide S16/S32/S32 Pro/S96 Dex, and click the Run button to start the experiment.

3.5 At the end of the automated extraction procedure, the RNA in well 5 of the single-sample reagent strip was aspirated and stored under appropriate conditions.

Appendix

Table 1: Total RNA extraction procedure of TGuide S16 fully automated nucleic acid extraction and purification instrument for universal use.

Step	Hole site	Name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Segments	Every time (sec)	Magneti-zation time(sec)	Cycle	Magnet speed (mm/sec)
1	6	Pipette beads	0.5	7	0	720	--	5	5	3	2	2
2	2	Collect beads	0.5	7	0	900	--	1	0	0	--	--
3	1	Lysis	5	8	0	900	--	1	0	0	--	--
4	2	Pipette beads	0.5	7	0	800	--	5	5	3	2	2
5	1	Bind	5	8	0	900	--	5	5	3	2	2
6	2	Wash 1	3	7	0	800	--	5	5	0	2	2
7	3	DNase I	12	3	0	710	--	1	5	0	2	2
8	2	Wash 2	5	7	0	800	--	5	5	3	2	2
9	4	Wash 3	3	7	0	800	--	5	5	0	2	2
10	6	Wash 4	3	7	6	720	--	5	5	0	2	2
11	5	Elution	5	7	0	100	45	5	5	5	2	2
12	6	Discard beads	0.5	5	0	720	--	1	0	0	--	--

Table 2: TGuide S32/S32 Pro fully automated nucleic acid extraction and purification instrument universal total RNA extraction procedure.

Step	Slot	Name	Waiting time (min)	Mixing time (min)	Magnetic suction time (min)	Mix speed	Volume (μl)	Temp. (°C)	Adsorption mode
1	6	Pipette beads	0	0.5	1	Fast	720	--	Cycle
2	2	Collect beads	0	0.5	0	Fast	800	--	--
3	1	Lysis	0	5	0	Fast	900	--	--
4	2	Pipette beads	0	0.5	1	Fast	800	--	Cycle
5	1	Blending	0	5	1	Fast	900	--	Cycle
6	2	Wash 1	0	3	1	Fast	800	--	Cycle
7	3	DNase I	0	12	1	Slow	710	--	Cycle
8	2	Wash 2	0	5	1	Fast	800	--	Cycle
9	4	Wash 3	0	3	1	Fast	800	--	Cycle
10	6	Wash 4	0	3	1	Fast	720	--	Cycle
11	5	Elution	6	5	2	Fast	100	45	Cycle
12	6	Discard beads	0	0.5	0	Fast	800	--	--

Table 3: TGuide S96 Dex fully automated nucleic acid extraction and purification instrument universal total RNA extraction procedure

Step	Well site	Name	Waiting time before mixing (mm:ss)	Mix speed	Mixing time (mm:ss)	Mixing mode	Liquid volume (μl)	Magnetic suction time (mm:ss)	Number of magnetic aspirations	Heating temperature (°C)	Pause after completion
1	6	Pipette beads	0	Fast	0:30	Normal mode	720	0:30	2	--	--
2	2	Collect beads	0	Fast	0:30	Normal mode	800	0:00	0	--	--
3	1	Lysis	0	Fast	5:00	Normal mode	900	0:00	0	--	--
4	2	Pipette beads	0	Fast	0:30	Normal mode	800	0:30	2	--	--
5	1	Bind	0	Fast	5:00	Normal mode	900	0:30	2	--	--
6	2	Wash 1	0	Fast	3:00	Normal mode	900	0:30	2	--	--
7	3	DNase I	0	Slow	12:00	Normal mode	710	0:30	2	--	--
8	2	Wash 2	0	Fast	5:00	Normal mode	900	0:30	2	--	--
9	4	Wash 3	0	Fast	3:00	Normal mode	800	0:30	2	--	--
10	6	Wash 4	0	Fast	3:00	Normal mode	720	0:30	2	--	--
11	5	Elution	6:00	Fast	5:00	Normal mode	100	0:30	3	45	--
12	6	Discard beads	0	Fast	0:30	Normal mode	720	0	0	--	--