TGuide Total RNA Whole Blood Kit

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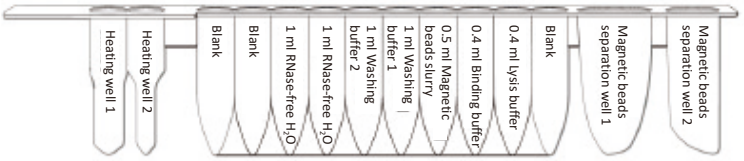
TGuide Total RNA Whole Blood Kit

Cat. No. OSR-M610-B

Kit Contents

Contents	OSR-M610-B (48 rxn)
Prepacked Reagent Cartridge (610)	48
Pipette Tips/Tip Caps	48
1.5 ml Sample Tubes (luer lock)	100
1.5 ml Centrifuge tubes	50
RNase-free DNase I (1500 Kunitz units)	1 tube
Buffer RDD	4 ml
RNase-free ddH_2O (tube)	1 ml
1×Red cell lysis buffer	250 ml
Buffer RBB	15 ml
Handbook	1

Reagent Cartridge:



Storage Conditions:

RNase-free DNase I, Buffer RDD and RNase-free ddH_2O (tube) are stored at 2-8°C for 12 months. Buffer RBB added with β-mercaptoethanol can be stored for one month at 2-8°C, and other reagents can be stored at room temperature (15-30°C) for 12 months.

Other Related Reagents

β-mercaptoethanol

Product Description:

TGuide Total RNA Whole Blood Kit is specially designed to extract high purity RNA from different whole blood samples using TGuide M16 Automated Nucleic Acid Extractor, with no protein and other impurities pollution. The kit contains reagents and consumables required for automatic RNA extraction by magnetic bead method, and the reagents are prepacked in sealed reagent cartridges. Unique embedded magnetic beads and fully automatic extraction process ensure RNA separation quickly and conveniently.

RNA isolated by this kit can be used in various downstream experiments such as RT-PCR, Real Time PCR, chip analysis, Northern blot, Dot blot, polyA screening, in vitro translation, RNase protection analysis and molecular cloning without purification.

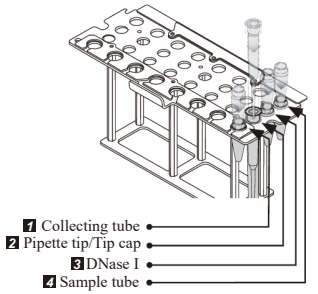
Product Features

Simple and fast: Ultrapure nucleic acid can be obtained in 73 min.

No contamination: The independent sealed prepacked reagent cartridge can avoid cross-contamination.

Safe and harmless: The kit and the operation process do not need to use organic solvents harmful to human body such as phenol and chloroform.

The Setting of the T-rack:



Note: Read this note before using this kit.

1. This kit must be combined with TGuide M16 Automatic Nucleic Acid Extractor.
2. This kit is suitable for fresh whole blood samples and blood samples stored with RNALock blood RNA stabilizer, but not frozen whole blood samples.

3. Before operation, β-mercaptoethanol is added to Buffer RBB to a final concentration of 1%, for example, 10 µl β- mercaptoethanol is added to 1 ml Buffer RBB. It is recommended to prepare the lysis buffer right before use. The prepared Buffer RBB can be stored at 2-8°C for one month. Buffer RBB may form precipitates during storage. If there is any precipitate, please heat the buffer to dissolve the precipitate before use.

Preparation of DNase I stock solution:

Dissolve DNase I dry powder (1,500 U) in 550 µl RNase-free ddH_2O, mix gently, and store at -30~-15°C after aliquoting (the stock solution can be stored for 9 months).

Note: DNase I stock solution thawed from -30~-15°C shall be stored at 2-8°C (can be stored for 6 weeks), and shall not be frozen again.

Preparation of DNase I working solution:

Add 7 volume of Buffer RDD to 1 volume of DNase I stock solution in a new RNase-free centrifuge tube, and mix gently.

Operation steps:

RNA purification from fresh whole blood:

1. Add 3 volume of 1×Red cell lysis buffer to 1 volume of human whole blood (for example, add 1,200 µl of 1×Red cell lysis buffer to 400 µl whole blood sample).

   Note: Due to the limited lysis strength of the subsequent lysis buffer, it is recommended to apply less than 5 ml of the initial sample amount.

2. Incubate on ice for 10-15 min and mix evenly for 2 times by vortex during incubation.
3. Centrifuge at 2,500 rpm (~500×g) for 3 min at 4**°C** and completely remove the supernatant.
4. Add 500 µl of 1×Red cell lysis buffer to leukocyte precipitate to resuspend cells.
5. Centrifuge at 2,500 rpm (~500×g) for 3 min at 4°C and completely remove the supernatant.
6. Add 200 µl of Buffer RBB to the leukocyte precipitate (please add β-mercaptoethanol before use), gently vortex or mix thoroughly with a pipette.

Note: If the precipitate is difficult to dissolve, up to 300 µl of Buffer RBB can be added.

7. Transfer the solution to a sample tube and place the sample tube in the well 4 of the T-rack.
8. Add 80 µl of DNase I working solution and 120 µl of RNase-free ddH_2O to another new 1.5 ml sample tube, and place the sample tube at the position of well 3 of the T-rack.
9. Run Program No. 610 (total RNA extraction program).

   Note: 60 µl elution volume is recommended.

RNA purification from blood sample preserved by RNALock blood RNA stablizer:

1. Add 3 volume of the RNALock to 1 volume of human whole blood for subsequent storage.

   Note: This step involves blood preservation. Please refer to the RNALock instruction for details.

   Since the precipitation obtained by centrifugation will increase after adding the blood RNA stabilizer, it is suggested to use 3 ml of the whole blood sample. Excessive amount will cause incomplete suspension by Buffer RBB.
2. Place the preserved sample at room temperature or warm to room temperature in a 37°C water bath, then centrifuge at 6,600 rpm (~4,000×g) for 10 min. Pipette out the supernatant, and take the precipitate for the following operations.
3. Add 2 volume of RNase-free ddH_2O to 1 volume of blood sample, pipette up and down to completely dissolve the precipitate.

   Note: The precipitate is difficult to dissolve in this step. Please pipette to mix until the precipitate is completely dissolved, otherwise the RNA yield will be reduced.
4. Add 200 µl of Buffer RBB to the dissolved sample (please add β-mercaptoethanol before use).

   Note: If the precipitate is difficult to dissolve, up to 300 µl of Buffer RBB can be added.
5. Transfer the solution to a sample tube and place the sample tube in the well 4 of the T-rack.
6. Add 80 µl of DNase I working solution and 120 µl of RNase-free ddH_2O to another new 1.5 ml sample tube, and place the sample tube at the position of well 3 of the T-rack.
7. Run Program No. 610 (total RNA extraction program).

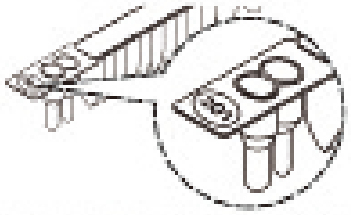
   Note: 60 µl elution volume is recommended.

Start program

Apply your specimen to TGuide after installing all necessary accessories.

Press START

After the Start button is pressed, the machine executes the calibration procedure, initializes, and moves all axes to the original position.



Enter the cartridge code and execute the program. The cartridge code is displayed on the prepacked reagent cartridge and the cover of the manual.

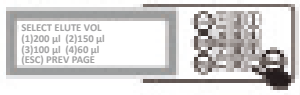
The above code is for demonstration purposes, please refer to the reagent cartridge you will actually purchase.

Confirm the cartridge code you entered again and press Enter to select the sample volume on the next page.

Select the sample volume

Confirm the sample volume.
Press Enter to enter the next page;
Press ESC to return to the Stand-By page.

In this step, check whether the cartridge rack and T-rack are in the work area.
Then press Enter to select the elution volume on the next page



Select elution volume

In this process, the green LCD indicator lights up and the heater starts to heat up to 65°C for the lysis step.
The TGuide LCD light is on at all times during the TGuide M16 program.
Don't open the door at this time, it will cause an emergency stop. You may lose your sample due to machine interruption.

When the program is completed, an alarm sound can be heard and the green LCD indicator goes out.