【Product Name】 MagPure Universal RNA Precast Kit (Auto Pure 96)

【Product Specification】96 Preps/Kit

【Intended Use】

This product is suitable for rapid extraction of RNA from tissue, cells, blood, s and other clinical samples. RNA can be used directly for RT-PCR, quantitative RT-PCR and so on.

【Principle】

The Kit combines the speed and efficiency of silica-based technology with the convenient handling of magnetic particles for purification of total RNA. Samples are lysed and RNA is purified from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet and DNA is removed by treatment with RNase-free DNase I. The magnetic particles are efficiently washed, and RNA is eluted in RNase-free water.

【Main Composition】

Cat.No	Precast Reagent	IVD3020-F-96
DNase Buffer		60 ml
DNase I		2 x 600 µl
RTL Lysis Buffer		80 ml
Buffer MCB		18 ml
96-Tip		1
Sample Plate (DW Plate)	500µl Buffer MCB	1
Wash Plate 1 (DW Plate)	700µl Buffer MW1 30µl MagPure RNA Partilces	1
DNase Plate	Empty	1
Wash Plate 2 (DW Plate)	700µl Buffer MW1	1
Wash Plate 3 (DW Plate)	900µl Buffer MW2	1
Elution Plate (DW Plate)	80µl RNase Free Water	1

【Storage conditions and validity】

DNase I should be shipped with iece pack and stored at -20°C after arrival. However, short-term storage (up to 1 week) at room temperature (15–25°C) does not affect its performance. The remaining kit components can be stored at room temperature (15–25°C) for 18 months.

【Applicable Instrument】

Nucleic Acid Extraction Machine such as Auto Pure 96 (Allsheng) or similar.

【Preparation before Use】

- (Optional) 2-mercaptoethanol can be added to an aliquot of RTL Lysis Buffer before use. Add 20µl 2-mercaptoethanol per 1mL RTL Lysis Buffer. This mixture can be stored for 2 weeks at room temperature
- Add 42 ml (96 Preps) isopropanol to the bottle of MCB.

【Part 1: Sample Preparation】

a. Cells Grown in Suspension (no more than 5 x 10^6 cells)

Pellet cells by centrifugation. loosen the cell pellet thoroughly by flicking the tube. Add 500µl of RTL Lysis Buffer and vortex vigorously.

b. Cells Grown in Monolayer (no more than 5 x 10^6 cells)

Lyse cells directly in a culture dish by adding 500µl of RTL Lysis Buffer and passing the cell lysate several times through a blue pipette tip. Always use more RTL Lysis Buffer if in the lysate is too viscous to aspirate with a pipette.

c. Animal Tissue (Do not use more than 20 mg Tissue)

Homogenize no more than 20mg tissue samples in 500µl RTL Lysis Buffer using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube and add RTL Lysis Buffer. After lysate, centrifuge at 14,000 x g for 3 minute at room temperature.

d. Plant Tissue (Do not use more than 100 mg Tissue)

Disruption Plant sample by liquid nitrogen and transfer up to 100mg power to 1.5ml Tube.Add 500µl RTL Lysis Buffer to the sample and mix well by vortexing vigorously. Centrifuge at 14,000 x g for 3

minute at room temperature.

e. **Whole Blood (Do not use more than 1.5ml blood)**

Separate the leukocytes cell from 0.5~1.5ml Whole blood. Resuspend the leukocytes pellets completely by 50µl Buffe PBS. Add 450µl of RTL Lysis Buffer and vortex vigorously to lyse.

f. **Trizol/MagZol Regeant (without chloroform extraction):**

Following Trizol/MagZol Reagent protocol, add 500µl MagZol Reagent to lyse the sample. After lysate, centrifuge at 12,000 x g for 10 minutes at 2-8°C.

g. **Trizol/MagZol Regeant (with chloroform extraction):**

Following Trizol/MagZol Reagent protocol, add 1ml Trizol/MagZol Reagent to lyse the sample. After lysate, add 200µl chloroform to the lysate and centrifuge at 12,000 x g for 15 minutes at 2-8°C.

【Part 2: Auto Pure 96 nucleic acid extractor operation】

1. Take out the required components of the kit. Inverting the Wash Plate 1 several times to re-suspend the magnetic beads. Remove the sealing bag and sealing film.
2. **Add 400~450µl the lysate or the supernatant into each well of Sample Plate.**
3. **Add 290µl DNase Buffer and 10µl DNase I into each well of DNase Plate.** DNase Buffer and DNase I can mix before adding in to the plate.
4. Place a 96 tip comb for deep well magnets on Wash Plate 1. Load the plates on the machine
5. Turn on the machine and start the IVD3020-F-96 protocol.
6. **Add 450µl Buffer MCB to the DNase plate during the dispense step.** Place the DNase plate back into the instrument and continue the program.
7. After the run is completed, remove the plates and store the purified total RNA at -20 °C.

Name	Plate	Mix time (min)	Mix 1-100%	Wait	Volume (ul)	Mix Speed (1-10)	Magnet (0-5)	Repeat (1-10)	Magnet Speed (1-10)	Stay (min)	Hover (min)	1st Step Magnet time	2nd step Magnet time	3rd step Magnet time
96-Tip	2	0	0	0	700									
Collect	2	0.5min	70%	0	700	7	3	1	5	0	0	3	3	3
Sample	1	5min	70%	0	950	7	3	2	5	0	0	5	5	5
Wash1	2	2min	70%	0	700	8	3	1	1	0	0	3	3	3
Dry	3	0min	70%	3min	600	8	0	0	0	0	0	0	0	0
DNase	3	12min	70%	0	300	8	0	0	0	0	0	0	0	0

Name	Plate	Mix time (min)	Mix 1-100%	Wait	Volume (ul)	Mix Speed (1-10)	Magnet (0-5)	Repeat (1-10)	Magnet Speed (1-10)	Stay (min)	Hover (min)	1st Step Magnet time	2nd step Magnet time	3rd step Magnet time
96-Tip	2	0	0	0	700									
ReBind	3	5min	70%	0	800	7	3	1	5	0	0	3	3	3
Wash 1	4	5min	70%	0	700	7	3	2	5	0	0	5	5	5
Wash2	5	2min	70%	0	900	8	3	1	1	0	0	3	3	3
Dry	8	0	70%	6min	600	8	0	0	0	0	0	0	0	0
Elute	8	5min	70%	0	800	7	3	1	5	0	0	3	3	3
Drop	5	1min	70%	0	800	7	0	0	0	0	0	0	0	0