Mag Beads Universal RNA Isolation Kit

【Product Name】

Mag Beads Universal RNA Isolation Kit

【Package Specifications】

50T/box（Art.No.FP101-50）；100T/box（Art.No.FP101-100）

【Intended Use】

Extraction of total RNA from ≤1 x 10^7 cultured cells, and ≤50mg animal tissues (liver, spleen, kidney, brain, etc.). It is also suitable for high-throughput nucleic acid automation extraction equipment such as workstations. The obtained RNA can be directly used for downstream experiments such as Northern Blot and in vitro translation.

【Detection Principle】

RNA binds to the surface of silica-based Magbeads under high salt and low pH conditions. After multiple washes to remove impurities such as proteins, RNA is eluted under low salt conditions, resulting in high-purity RNA.

【Main Components】

Components	FP101-50	FP101-100
Bead Suspension	1.1 mL	2×1.1 mL
Buffer PLB	30 mL	55 mL
Buffer PL	15 mL	25 mL
DNase I	550 µL	2×550 µL
DNase Buffer	15 mL	30 mL
Buffer W1A*	30 mL	66 mL
Buffer W2R*	15 mL	30 mL
Buffer VLF	50 mL	100 mL
RNase Free Water	5 mL	10 mL

【Self-provided Equipment and Reagents】

1．Equipment: Nucleic acid extractor , 2.2mL 96-well deep plate (U-bottom), magnetic bar sleeve, centrifuge tubes, magnetic rack, vortex shaker, pipettor, heating shaker, etc.;

2．Reagents: Absolute ethanol.

【Storage Conditions and Shelf Life】

Except for DNase I, this product can be stored at room temperature (15~25°C) for 12 months. DNase I can be shipped at room temperature and should be stored at -20°C upon receipt. Since RNase Free Water does not contain antimicrobial agents, bacterial or fungal contamination may occur when stored or handled at room temperature. It is recommended to aliquot and store at 2~8°C to reduce contamination.

【Precautions】

1．Before using Buffer W1A/W2R, dilute with absolute ethanol as indicated on the bottle label.

2．Avoid repeated freeze-thaw cycles of samples.

3．Magnetic beads should not be frozen, and the magnetic bead suspension should be thoroughly mixed before use.

4．Wear protective equipment before operation. In case of accidental skin or eye contact with reagents, immediately rinse with water for 5-10 minutes.

5．Before each use, check if the lysis solution has precipitated. If so, re-dissolve at 60°C.

【Manual Operation Steps using Centrifuge Tubes】

1. Sample Processing and Lysis

1) Sample Pre-processing:

A. Grind the tissue sample with liquid nitrogen, weigh ≤20mg of tissue sample, add 500μL Buffer PL, vortex at high speed for 20~60s to disperse the sample, and incubate at room temperature for 5 minutes.

Note: Tissue samples can be homogenized using tools such as liquid nitrogen grinding, mechanical/glass homogenizer, bead mill, etc. Then centrifuge at 14,000 x g for 5 minutes. Proceed to step 2).

B. Take the precipitate of 10^6 cells, add 500μL Buffer PLB, vortex or pipette to disperse the cells, and incubate at room temperature for 5 minutes.

Note: If the sample is a precipitate of white blood cells, the volume of blood for obtaining the precipitate should be less than 1.5mL.

2) Centrifuge at 13,000 x g for 15 minutes, transfer 450~500μL of the supernatant to a new centrifuge tube for later use.

2. Binding:

Add 20μL of magnetic bead suspension and 450μL Buffer VLF, vortex thoroughly. Incubate at room temperature for 5 minutes, and invert several times during this period.

3. Magnetic Separation: Place the centrifuge tube on the magnetic rack and let it stand for 30 seconds until the magnetic beads are fully adsorbed, then carefully discard the liquid.

4. Wash 1: Remove the centrifuge tube from the magnetic rack, add 600μL of Buffer W1A (ensure ethanol has been added), vortex mix for 1 minute. Place the centrifuge tube on the magnetic rack and let it stand for 30 seconds until the magnetic beads are fully adsorbed, then carefully discard the liquid.

5. Ethanol Removal by Centrifugation: Briefly centrifuge, place the centrifuge tube on the magnetic rack and let it stand for 10 seconds, then remove the residual liquid. Open the tube cap and let it stand for 3 minutes (this step cannot be omitted as excessive ethanol residue may inhibit subsequent experiments).

6. Add 10μL of DNase I and 70μL of DNase Buffer to the centrifuge tube, gently pipette to mix the magnetic beads. Incubate at room temperature for 15 minutes.

7. Rebinding: Add 600μL of Buffer VLF, vortex mix for 1 minute, and let it stand at room temperature for 3 minutes.

8. Magnetic Separation: Place the centrifuge tube on the magnetic rack and let it stand for 30 seconds until the magnetic beads are fully adsorbed, then carefully discard the liquid.

9. Wash 2: Remove the centrifuge tube from the magnetic rack, add 600μL of Buffer W2R (ensure ethanol has been added), vortex mix for 1 minute. Place the centrifuge tube on the magnetic rack and let it stand for 30 seconds until the magnetic beads are fully adsorbed, then carefully discard the liquid.

10. Wash 3: Repeat step 5 once.

11. Magnetic Separation and Ethanol Removal: Briefly centrifuge, place the centrifuge tube on the magnetic rack and let it stand for 10 seconds, then remove the residual liquid (this step cannot be omitted as excessive ethanol residue may inhibit subsequent experiments); place the centrifuge tube on the magnetic rack, open the lid, and air dry at room temperature for 5-10 minutes (excessive drying time may cause difficulties in elution).

12. Elution: Remove the centrifuge tube, add 100μL of RNase Free Water, vortex mix, and shake at 1500rpm at 55°C for 5 minutes (or use a 55°C water bath, vortex mix 3~4

times during this period).

13. RNA Transfer: Place the centrifuge tube on the magnetic rack, let it stand for 2 minutes to adsorb the magnet, then transfer the RNA solution to a new centrifuge tube and store at -80°C.

【Operation Steps for QP-AUT-16/32 Channel Nucleic Acid Extractor】

1. Sample Handling and Lysis

Refer to the steps outlined in the "Manual Centrifuge Tube Operation Steps".

2. Sample Preparation:

Add the specified amounts into each corresponding well of the 96-well plate as shown in the table below, enabling the simultaneous processing of 16/32 samples.

Note: The "Manual Centrifuge Tube Operation Steps" are assumed to be previously provided and detailed in the document.

Position	1/7	2/8	3/9	4/10	5/11	6/12
Reagent	Binding Buffer（20μL）+Buffer VLF（450μL）	Buffer W1A（600μL）	DNase I（10μL）+ DNase Buffer（70μL）；After the pause+Buffer VLF（450μL）	Buffer W2R（600μL）	Buffer W2R（600μL）	RNase Free Water（100μL）

3. Automated Extraction:

Transfer the prepared supernatant (450-500μL) from the above process to the wells containing magnetic beads and ethanol (i.e., well positions 1/7 for the 32-channel extractor). Then, place the prepared 96-well sample plate into the fully automatic nucleic acid extractor in sequence, insert the magnetic rod sleeve, open the operation program of the instrument, select the corresponding program, click "Run," and initiate the extraction process.

4. Nucleic Acid Transfer:

After the instrument operation is complete, transfer the eluate from the 6th/12th columns of the 96-well deep plate to clean centrifuge tubes free of nucleases.

The parameters for the 32-channel nucleic acid extractor (QP-AUT-32) program are set as follows

Step	Site	Name	Waiting time(min)	Mixing time（min）	Suction time(sec)	Volume (μL)	Mixing velocity	Temperature
1	1	Lysis Binding	0	5	60	1000	2	OFF
2	2	Washing 1	0	2	60	600	3	OFF
3	3	Binding	0	15	0	80	3	OFF
Suspend								
4	3	Binding	0	5	60	550	3	OFF
5	4	Washing2	0	1	60	600	3	OFF
6	5	Washing3	0	1	60	600	3	OFF
7	6	Elution	2	5	60	100	3	60℃
8	5	Abandon beads	0	1	0	600	2	OFF

【Operation Steps for QP-AUT-96 Channel Nucleic Acid Extractor】

1. Sample Handling and Lysis

Refer to the steps outlined in the "Manual Centrifuge Tube Operation Steps".

2. Sample Preparation

Add the specified amounts into each corresponding well of the 96-well plate as shown in the table below, enabling the simultaneous processing of 96 samples.

Position	1	2	3	4	5	6
Reagent	Bead Suspension（20μL）+Anhydrous ethanol（350μL）	Buffer W1A（600μL）	DNase I（10μL）+ DNase Buffer（70μL）; After the pause+Buffer VLF（450μL）	Buffer W2R（600μL）	Buffer W2R（600μL）	RNase Free Water（100μL）

3. Automated Extraction:

Transfer the processed supernatant to the 96-deep well plate in workstation 1. Then, sequentially place the prepared 96-well sample plate into the nucleic acid extractor, insert the magnetic rod sleeve, open the instrument's operating program, select the corresponding program, click "Run," and initiate the extraction process.

4. Nucleic Acid Transfer:

After the instrument operation is complete, either seal the eluate from the 6th workstation or transfer it directly to clean centrifuge tubes free of nucleases, and store it at -20°C for future use.

The parameters for the 96-channel nucleic acid extractor (QP-AUT-96) program are set as follows

Procedure	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6	Step 7	Step 8
Station	1	2	3	3	4	5	6	5
Waiting time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:00	00:00:00	00:02:00	00:00:00
Mixed model	2	3	3	3	3	3	3	2
Mixing time	00:05:00	00:02:00	00:15:00	00:05:00	00:01:00	00:01:00	00:05:00	00:00:30
Suspend	No	No	Yes	No	No	No	No	No
Suction time	00:01:00	00:01:00	00:00:00	00:01:00	00:01:00	00:01:00	00:01:00	00:00:00
Volume	1000μL	600 μL	80 μL	550 μL	600 μL	600 μL	100 μL	600 μL
Temperature	—	—	—	—	—	—	60℃	—

【Basic Information】

Version Number: 1.1

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