

Product components

● For S-16/S-48 Fully Automated Nucleic Acid Extraction System

Components	Component number	Size	
		16	RXN
Mag Buffer RLA	RM30286	11	mL
AFTMag Quick Tissue/Cell RNA Extraction Reagents for Animal (Prepackaged)	RM30296	16	RXN
RNase-free Water *	RM30295	2	mL
DNase I, RNase-free (5,000 U/mL)	RM21312	80	µL
DNase I Buffer	RM30588	2	× 1 mL
8-Well Mag-rod Sleeve	RM30277	2	pk

*50 µL of elution buffer RNase-free Water is pre-dispensed in the prepackaged 96-well plate. An additional separate tube of 2 mL RNase-free Water is provided for use as needed.

● For N-96 Fully Automated Nucleic Acid Extraction System

Components	Component number	Size	
		96	RXN
Mag Buffer RLA	RM30286	55	mL
Mag Buffer BD	RM30287	96	RXN
Mag Buffer WA1	RM30288	96	RXN
Mag Buffer WA2 & Beads	RM30297	96	RXN
Mag Buffer WA3	RM30338	96	RXN
RNase-free Water	RM30295	96	RXN
RNase-free Water *	RM30295	2	mL
DNase I, RNase-free (5,000 U/mL)	RM21312	2	× 220 µL
DNase I Buffer	RM30588	12	mL
96-Well Mag-rod Sleeve	RM30283	1	pk
96 Deep-Well Plate, U-Bottom, Square Well, 2.2 mL	RM30278	1	pk

*50 µL of elution buffer RNase-free Water is pre-dispensed in the prepackaged 96-well plate. An additional separate tube of 2 mL RNase-free Water is provided for use as needed.

Product Description

This kit is a nucleic acid extraction kit based on bio-nanomagnetic beads with high-specificity binding. The main principle involves utilizing the functional groups on the surface of the bio-magnetic beads to enrich nucleic acids from the sample lysate onto the surface of the beads. The proteins and other impurities are removed with a wash solution, followed by separation of the beads using a magnetic separation device. DNase I digestion is then employed to remove DNA, allowing for the rapid separation and purification of RNA. The entire process does not require the use of harmful substances such as phenol, chloroform, or β-mercaptoethanol, making it safe and non-toxic. Moreover, the paramagnetic bio-nanomagnetic beads are suitable for high-throughput automated extraction. The extracted products can be directly used in downstream experiments such as RT-PCR, qRT-PCR, Northern Blot, next-generation sequencing library preparation, and molecular cloning.

Applicable samples

This kit is mainly applicable to total RNA extraction from various animal cultured cells (10^3 - 10^7 cells) as well as fresh and frozen animal tissues..

Storage

DNase I: Store at -20°C;

DNase I Buffer: Store at 4°C;

Other reagents: Store at room temperature (10-30°C).

Precautions

- RNase contamination should be avoided throughout the RNA extraction experiments with this kit:
 - Take care to change new gloves frequently to avoid degradation of sample RNA due to RNase on the skin surface.
 - Use plastics such as RNase-free tips.
 - If other glassware is required for the extraction process, it can be baked at 150°C for 4-6 h. The glassware can be used for the extraction process.
- RNA yield and purity issues:
 - The beads need to be well dried or the A260/A230 ratio will be affected.
 - The volume of elution buffer can be adjusted appropriately according to the sample amount. For extracting trace cells, the elution volume can be reduced to increase concentration. However, an elution volume below 30 μ L will prevent complete resuspension of the beads, leading to insufficient elution and reduced RNA yield.
 - For cellular RNA extraction, please use fresh samples as much as possible and avoid repeated freezing and thawing of frozen samples.
 - Extraction of total RNA from animal tissues was performed using liquid nitrogen as much as possible to treat ultra-low temperature preserved samples.
- When extracting RNA from cells $>5 \times 10^6$, the lysate homogenate becomes viscous after adding the lysis buffer. To facilitate pipetting, the tip end can be cut off, or the lysate homogenate can be poured directly into the corresponding wells of a deep-well plate for the binding reaction. Subsequent extraction is unaffected.
- For tissues high in protein content (e.g., heart, muscle), fat, brain tissue, and trace cell samples, prepare Proteinase K (ABclonal, RP02503LQ) separately.
- For blood samples, prepare Red Blood Cell Lysis Buffer, Proteinase K (ABclonal, RP02503LQ), and DNase I, RNase-free (5,000 U/mL) (ABclonal, RK20549) separately.

Product Performance Indicators

- Extraction Yield from 293F Cells:

Cell Input	1000	2000	5000	1×10^4	1×10^5	1×10^6	2×10^6	5×10^6	1×10^7
RNA yield (μ g)	0.1	0.16	0.27	0.64	5	30	45	112	183

Extraction Yield from Mouse Tissues:

Tissue Type	Heart	Liver	Spleen	Lung	Kidney	Brain	Fat	Muscle	Testis	Tail	Skin
RNA yield (μ g/10 mg tissue)	2-8	60-90	70-120	20-35	35-50	5-12	5-12	2-5	20-30	2-5	5-10

- Extraction Purity:

A260/A280 ratio >1.8 , A260/A230 ratio typically >1.7

For trace cells (10^3 - 10^4 cells), A260/A230 ratio >1.0

- Nucleic Acid Integrity:

Agarose gel electrophoresis: Clear 28S and 18S bands without significant smearing, with 28S/18S intensity ratio >2

Capillary electrophoresis: RQN value >8

Operating Instructions

Experimental Preparation

According to the number of samples, prepare the corresponding volume of DNase I reaction mix (usage per reaction shown in Table 1). Dispense into the corresponding wells of the 96-well plate or place on ice/4°C refrigerator for later use (manual extraction). The DNase I reaction mix should be prepared fresh to avoid reduced enzyme activity.

Table 1. DNase I Reaction Mix Preparation

Components	For Cells/Tissues*	For Blood (1 mL)**	For Blood (1.5 mL)**
DNase I, RNase-free (5,000 U/mL)	4 µL	10 µL	12 µL
DNase I Buffer	96 µL	90 µL	88 µL

***Note:** Briefly centrifuge DNase I before use to collect liquid at tube bottom. For $\leq 10^5$ cells or tissues with low DNA content, reduce DNase I to 2-3 µL and adjust DNase I Buffer to 98-97 µL accordingly.

****Note:** For blood samples, additional DNase I, RNase-free (5,000 U/mL) (ABclonal, RK20549) is required as the kit's standard amount is insufficient.

Sample Lysis and Homogenization

1. Suspension Cells

Transfer appropriate amount of fresh cell suspension ($\leq 10^7$ cells) to a 1.5 mL tube. Centrifuge at $600 \times g$, 4°C for 5 min. Discard supernatant completely. Add recommended volume of lysis buffer **Mag Buffer RLA** (refer to Table 2). Resuspend cells by pipetting or vortex until the homogenate is clear.

2. Adherent Cells

Discard the culture medium supernatant from monolayer adherent cells. Wash with 1X PBS to remove residual medium to avoid affecting downstream reactions. Lysis buffer can then be added directly to the culture dish.

(1) Cell count $\leq 10^7$: Add the recommended volume of lysis buffer **Mag Buffer RLA** (refer to Table 2). Pipette repeatedly to detach and lyse cells.

(2) Cell count $>10^7$: Digest with trypsin, resuspend in PBS, take a suspension containing a certain number of cells, and lyse according to the suspension cell method.

(3) Adherent cells in 96-well plate: After discarding the medium or PBS wash supernatant completely, add 110 µL/well of freshly prepared lysis mix (prepared by mixing 100 µL **Mag Buffer RLA** + 10 µL **Proteinase K (20 mg/mL)** per well*. Prepare immediately before use. Prepared lysis mix must be added to cells within 30 minutes). Pipette repeatedly to detach and lyse cells.

***Note:** Add 10 µL of Proteinase K (20 mg/mL) per sample, independent of the volume of Mag Buffer RLA used.

Table 2. Lysis Buffer Volumes for Different Cell Inputs

Cell Input	Mag Buffer RLA
$\leq 10^5$	300 µL
$10^5 - 10^7$	500 µL
Adherent cells in a 96-well plate	100 µL

3. Trizol-preserved Cells

For fresh cultured cells, add 1 mL Trizol to 10^5 - 10^7 cell pellet. Mix well and freeze at -80°C. For extraction, take 500 µL Trizol-preserved homogenate, add 200 µL **Mag Buffer RLA**, mix well, then transfer to 300 µL Mag Buffer BD.

Note: For standard prepackaged reagents, the volume of Mag Buffer BD is 400 µL. Remove 100 µL, and retain 300 µL for use.

4. Animal Tissue (5-30 mg)

Table 3. Recommended Tissue Input Amounts

Tissue Type	Liver	Spleen	Lung	Kidney	Heart	Brain	Muscle
Recommended Input	≤20 mg	≤20 mg	≤30 mg	≤30 mg	10-40 mg	5-40 mg	10-40 mg

Note: If extracting a tissue type with no reference range for RNA abundance for the first time, it is recommended to start with a sample amount of 10 mg.

(1) Dry grinding

- a. Grind the tissue to powder with a mortar in a low-temperature environment maintained by liquid nitrogen. Or take about 10-20 mg of tissue cut into strips or slices and put it into a 2 mL centrifuge tube, add three 5 mm steel balls, and immediately place it in liquid nitrogen for cooling. Grind at 60 Hz for 30 seconds (with a liquid nitrogen pre-cooled metal module) until the tissue is powdery.

Note: Confirm that the tissue is sufficiently ground into powder, if there are still a few lumps of tissue, increase the number of grinding times as appropriate, and pre-cool the metal module with liquid nitrogen again for each grinding (multiple short grinding cycles prevent sample warming and degradation).

- b. Add 500 μ L **Mag Buffer RLA** (if the temperature is too low the liquid may freeze; shake the tube to melt it quickly). Vortex immediately for 2 min until no visible lumpy/flocculent tissue particles remain. (For tissues high in protein content like heart, muscle, fat, and brain tissue, add 0.2 mg Proteinase K/sample, i.e., 10 μ L **Proteinase K (20 mg/mL)** per sample after lysis. Mix thoroughly and incubate for 2-3 min). Centrifuge at 16,000 \times g for 5 min at 4°C, and take the supernatant for subsequent extraction.

(2) Wet grinding

Weigh about 10-20 mg of tissue block cut into strips or slices and put it into a 2 mL centrifuge tube, immediately add 500 μ L **Mag Buffer RLA** and three 5 mm steel beads, and grind at 60 Hz for 30 s. (For tissues high in protein content like heart, muscle, fat, and brain tissue, add 0.2 mg Proteinase K/sample, i.e., 10 μ L **Proteinase K (20 mg/mL)** per sample after lysis. Mix thoroughly and incubate for 2-3 min). Centrifuge at 16,000 \times g for 5 min at 4°C, and take the supernatant for subsequent extraction.

(3) Tissue Stored in Preservation Solution

Take out the tissue from the preservation solution, dry the liquid with clean absorbent paper, cut it into strips or slices of tissue blocks, and then perform dry grinding or wet grinding treatment according to normal tissues.

5. Frozen Section Tissue (≤ 10 slices, slice thickness 4-20 μ m)

Place frozen tissue sections into a centrifuge tube pre-cooled in liquid nitrogen. Immediately add 500 μ L **Mag Buffer RLA**. Vortex vigorously or pipette repeatedly to mix until no visible flocculent precipitate remains. Centrifuge at 16,000 \times g for 5 min at 4°C, and take the supernatant for subsequent extraction.

Note: For tissues with no reference range, it is recommended to start with a section thickness of 10 μ m and 2 sections for the initial extraction, then adjust based on the RNA yield.

6. Fresh Blood

(1) Red blood cell lysis: Take 1-1.5 mL fresh whole blood, add 3 volumes (3-4.5 mL) **RBC Lysis Buffer**. Mix gently by inversion and incubate on ice for 15 min, gently inverting twice during incubation (after RBC lysis, the solution should be clear and transparent). Centrifuge at 450 \times g for 10 min at 4°C. Discard supernatant.

(2) White blood cell collection: Add 2 volumes (2-3 mL) **RBC Lysis Buffer** to pellet. Resuspend gently. Centrifuge at 450 \times g for 10 min at 4°C. Discard supernatant.

(3) White blood cell lysis: Add 500 μ L **Mag Buffer RLA**. Vortex. Add 60 μ L **Proteinase K (20 mg/mL)**. Vortex for 30 s-1 min until no cell clumps.

Automated Extraction

1. Turn on the power of the instrument, wait until the instrument completes the self-test, set the program parameters according to Table 5-6.
2. (Omit this step for prepackaged reagents) For non-prepackaged reagents, please pre-package each component (shake well before use) according to the following table and make proper marks.

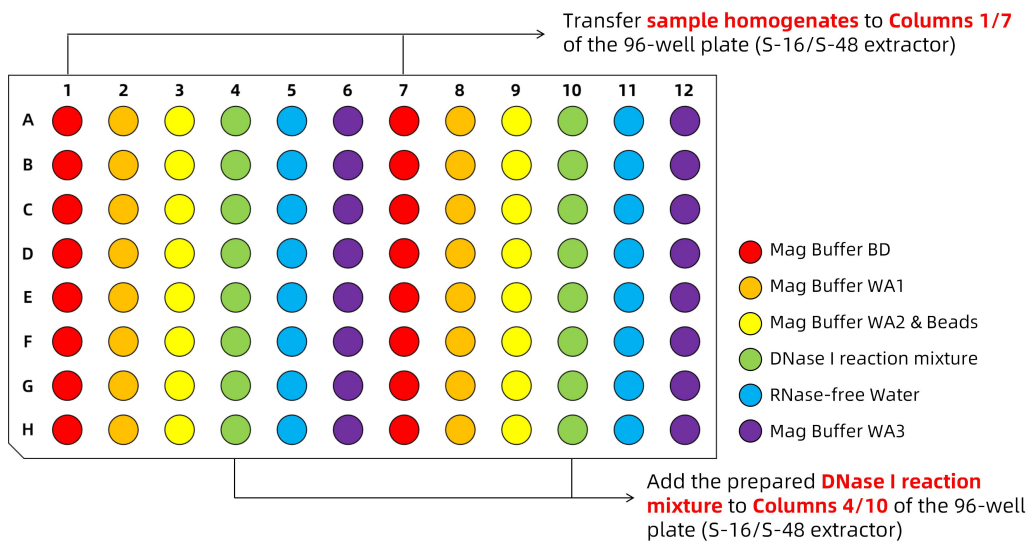
Table 4. Reagent Dispensing Volumes and Positions

Components	Volume per well	16 RXN - Column	96 RXN - Station
Mag Buffer BD	400 µL	Column 1/7	Station 1
Mag Buffer WA1	800 µL	Column 2/8	Station 2
Mag Buffer WA2 + Mag Beads A	800 µL+10 µL	Column 3/9	Station 3
DNase I Reaction Mixture	100 µL	Column 4/10	Station 4
Mag Buffer WA3	800 µL	Column 6/12	Station 5
RNase-free Water	30-100 µL	Column 5/11	Station 6

Note: The volume of RNase-free Water can be adjusted according to the downstream experiments on the concentration of RNA products, but the actual elution volume should not be less than 30 µL, otherwise the elution is not sufficient.

3. For pre-packaged reagents, gently flick the plate to collect reagents and beads at the bottom (or use a plate centrifuge at 500 × rpm for 1 min). Carefully peel off the sealing film (avoid vibration to prevent liquid splashing). Add the prepared **DNase I Reaction Mixture** to **Column 4/10 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 4 (N-96 extractor)**.
4. Process samples according to the sample lysis and homogenization protocol and transfer **sample homogenates** to **Column 1/7 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 1 of 96 RXN (N-96 extractor)**.
5. Place the 96-well plate into the corresponding station of the instrument, insert the magnetic rod sleeve, close the cabin door, and start running the program.
6. At the end of the program, the instrument will stop automatically, and the extracted nucleic acid samples are in **Column 5/11 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 6 (N-96 extractor)**, transfer the samples to clean RNase-free centrifuge tubes, and store them at -20°C for short-term storage and -80°C for long-term storage.

Reagent Layout Diagram



Extraction Programs for Different Instrument Models

Table 5. S-16/S-48 Fully Automated Nucleic Acid Extractor Reference Program

Step	1	2	3	4	5	6	7	8
Station	3	1	2	3	4	6	5	3
Wait Time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:15	00:00:00	00:04:00	00:00:00
Mix Mode	M1	M1 (M2)^a	M2 (M3)^a	M2 (M3)^a	M1	M2 (M3)^a	M1	M1
Mix Time	00:00:00	00:05:00 (00:10:00)^a	00:02:00	00:02:00	00:15:00	00:02:00	00:05:00	00:00:10
Pause	No	No	No	No	No	No	No	No
Mag Time	00:00:30	00:00:30	00:00:30	00:00:30	00:01:00	00:01:00	00:00:30	00:00:00
Volume	810 µL	900 µL (950 µL)^a	800 µL	810 µL	100 µL	800 µL	100 µL ^c	810 µL
Temperature ^b	--	25°C	--	--	--	--	60°C (70°C)^a	--

Note: a. Parameters in bold parentheses are for blood samples.

b. In the S-16/S-48 slot, Stations 1 and 5 corresponding to the 96-well plate have heating modules.

c. Set the instrument elution volume parameter according to the actual elution volume used. Elution volume should be ≥ 30 µL.

Table 6. N-96 Fully Automated Nucleic Acid Extractor Reference Program

Step	1	2	3	4	5	6	7	8
Station	3	1	2	3	4	5	6	3
Wait Time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:15	00:00:00	00:04:00	00:00:00
Mix Mode	M1	M1 (M2)^a	M2 (M3)^a	M2 (M3)^a	M1	M2 (M3)^a	M1	M1
Mix Time	00:00:00	00:05:00 (00:10:00)^a	00:02:00	00:02:00	00:15:00	00:02:00	00:05:00	00:00:10
Pause	No	No	No	No	No	No	No	No
Mag Time	00:00:30	00:00:30	00:00:30	00:00:30	00:01:00	00:01:00	00:00:30	00:00:00
Volume	810 µL	900 µL (950 µL)^a	800 µL	810 µL	100 µL	800 µL	100 µL ^c	810 µL
Temperature ^b	--	25°C	--	--	--	--	60°C (70°C)^a	--

Note: a. Parameters in bold parentheses are for blood samples.

b. In the N-96 slot, Stations 1 and 6 have heating modules.

c. Set the instrument elution volume parameter according to the actual elution volume used. Elution volume should be ≥ 30 µL.