AFTMag Quick Tissue/Cell RNA Extraction Kit for Animal

Cat. No. : RK30155

Product components

Components	Component number	Size-1 20 RXN	Size-2 100 RXN	Storage
Mag Buffer RLA	RM30286	11 mL	55 mL	RT
Mag Buffer BD	RM30287	10 mL	45 mL	RT
Mag Buffer WA1	RM30288	20 mL	90 mL	RT
Mag Buffer WA2	RM30289	20 mL	90 mL	RT
Mag Buffer WA3	RM30338	20 mL	90 mL	RT
Mag Beads A	RM30292	220 μL	1.1 mL	RT
DNase I, RNase-free (5,000 U/mL)	RM21312	80 μL	2 × 220 μL	-20°C
DNase I Buffer	RM30588	2 × 1.25 mL	12 mL	4°C
RNase-free Water	RM30295	4 mL	20 mL	RT

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 the extraction of total RNA from various animal cultured cells with a cell count of 10^3-10^7, as well as fresh and frozen animal tissues.

Storage

DNase I, RNase-free (5,000 U/mL): Store at -20°C;

DNase I Buffer: Store at 4°C;

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

Precautions

1. RNase contamination should be avoided throughout the RNA extraction experiments with this kit:
   (1)Take care to change new gloves frequently to avoid degradation of sample RNA due to RNase on the skin surface.
   (2)Use plastics such as RNase-free tips.
   (3)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.
2. RNA yield and purity issues:
   (1)The beads need to be well dried or the A260/A230 ratio will be affected.
   (2)The elution volume can be appropriately adjusted according to the sample volume, and the elution volume can be appropriately reduced and the concentration can be increased when extracting trace amounts of cells, but an elution volume lower than 30 μL will result in the magnetic beads not being able to be completely resuspended, the elution will be insufficient, and the RNA yield will be reduced.

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(3)For cellular RNA extraction, please use fresh samples as much as possible and avoid repeated freezing and thawing of frozen samples.

(4)Extraction of total RNA from animal tissues was performed using liquid nitrogen as much as possible to treat ultra-low temperature preserved samples.

3. When extracting RNA from cells more than 5×10^6 cells, the lysis homogenate is sticky after lysis with lysate, so you can choose to cut off the tip of the pipette tip for easy sample aspiration, or pour the lysis homogenate into the corresponding wells of the deep-well plate directly for binding reaction. Subsequent extraction is not affected.
4. For tissues high in protein content (e.g., heart, muscle), fat, brain tissue, and trace cell samples, prepare Proteinase K (ABclonal, RP02503LQ) separately.
5. 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

1. Extraction Yield from 293F Cells:

Cell Input	1000	2000	5000	1 x 10^4	1 x 10^5	1 x 10^6	2 x 10^6	5 x 10^6	1 x 10^7
RNA yield (μg)	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 (μg/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

2. 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

3. 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 ≤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 (≤10^7 cells) to a 1.5 mL tube. Centrifuge at 600 × g, 4°C for 5 min.

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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 ≤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
≤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℃. 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 µL **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 µL **Proteinase K (20 mg/mL)** per sample after lysis. Mix thoroughly and incubate for 2-3 min). Centrifuge at 16,000 × g for 5 min at 4°C, and take the supernatant for subsequent extraction.

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(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 µL **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 µL **Proteinase K (20 mg/mL)** per sample after lysis. Mix thoroughly and incubate for 2-3 min). Centrifuge at 16,000 × 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 µm)**

Place frozen tissue sections into a centrifuge tube pre-cooled in liquid nitrogen. Immediately add 500 µL **Mag Buffer RLA**. Vortex vigorously or pipette repeatedly to mix until no visible flocculent precipitate remains. Centrifuge at 16,000 × 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 µm 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 × 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 × g for 10 min at 4°C. Discard supernatant.

(3)White blood cell lysis: Add 500 µL **Mag Buffer RLA**. Vortex. Add 60 µL **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 Mix	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 Mix** 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)**.

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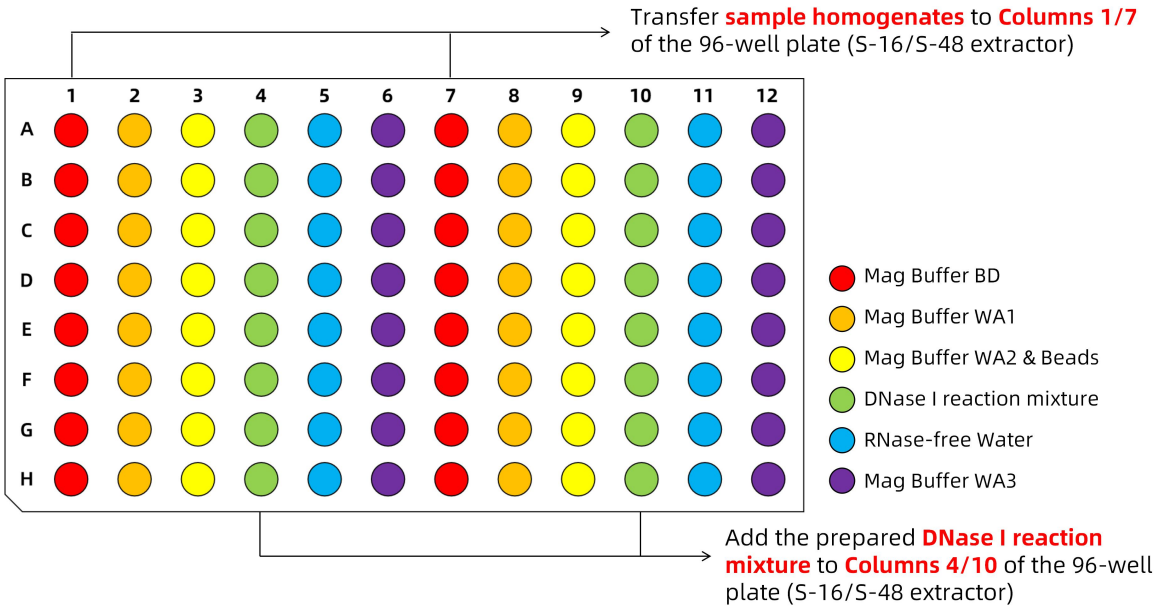
4. Process samples according to the sample lysis and homogenization protocol and transfer **sample homogenates** to **Column 1/7 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 1 of 96 RXN (N-96 extractor)**.
5. Place the 96-well plate into the corresponding 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℃ for short-term storage and -80℃ for long-term storage.

Reagent Layout Diagram



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Manual extraction

1. According to the sample lysis and homogenization protocol, different samples are processed to obtain the lysed sample homogenate.
2. Add 400 μL of **Mag Buffer BD**, invert and mix 6-8 times, then add 10 μL of **Mag Beads A** (be sure to mix before use) and vortex and mix for 5 min.

   Note: When the number of cells or the amount of tissue samples is higher, the amount of nucleic acid is higher, this step will produce flocculent precipitation after adding Mag Buffer BD, which is a normal phenomenon.
3. Centrifuge tubes are briefly centrifuged for 5 s (to avoid liquid residue on the tube cap) and then placed on a magnetic stand. Let it stand for 10 s. After the magnetic beads are completely adsorbed by the magnetic stand, aspirate and discard the liquid in the tube.
4. Take the centrifuge tube out from the magnetic stand, add 800 μL of **Mag Buffer WA1**, vortex and mix for 2 min. Briefly centrifuge the centrifuge tube for 5 s and then place it on the magnetic stand. Let it stand for 10 s. After the magnetic beads are adsorbed and aggregated, aspirate and discard the liquid in the tube.
5. Take the centrifuge tube out from the magnetic stand, add 800 μL of **Mag Buffer WA2**, vortex and mix for 2 min. Briefly centrifuge the centrifuge tube for 5 s and then place it on the magnetic stand. Let it stand for 10 s. After the magnetic beads are adsorbed and aggregated, aspirate and discard the liquid in the tube. Open the cap and let it air dry for 2 min.
6. Add 100 μL of prepared **DNase I Reaction Mix** directly into the centrifuge tube, pipette and mix to resuspend the magnetic beads, and place it at room temperature for 15 min (during this period, use your finger to flick the bottom of the centrifuge tube every 5 min to fully mix the magnetic beads and the reaction solution). After digestion is completed, briefly centrifuge the centrifuge tube for 5 s and then place it on the magnetic stand. Let it stand for 10 s. After the magnetic beads are adsorbed and aggregated, aspirate and discard the liquid in the tube.
7. Take the centrifuge tube out from the magnetic stand, add 800 μL of **Mag Buffer WA3**, vortex and mix for 2 min. Briefly centrifuge the centrifuge tube for 5 s and then place it on the magnetic stand. Let it stand for 10 s. After the magnetic beads are adsorbed and aggregated, aspirate and discard the liquid in the tube.
8. There is no need to take the centrifuge tube out from the magnetic stand. Open the cap and let it stand at room temperature for 4 min. After the magnetic beads are fully dried, add 30-100 μL of **RNase-free Water** to rinse the magnetic beads on the centrifuge tube wall to the bottom of the tube. Heat and shake and mix at 60℃ for 5 min (Or heat for 5 min, and gently flick the wall of the EP tube every minute to keep the magnetic beads dispersed). Briefly centrifuge the centrifuge tube and then place it on the magnetic stand. Let it stand for 10 s. After the magnetic beads are adsorbed and aggregated, carefully aspirate the liquid in the tube (which is the RNA sample) and transfer it to clean RNase-free centrifuge tubes. Store them at -20℃ for short-term storage and -80℃ for long-term storage.

   Note: a. To prevent residual liquid from affecting subsequent washing or elution steps, briefly centrifuge the tube for 5 s prior to magnetic separation to remove any liquid retained on the tube cap; b. During vortex-mixing, apply sufficient force to fully resuspend the magnetic beads and ensure they are thoroughly dispersed.

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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℃	--	--	--	--	60℃ **(70℃)**[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℃	--	--	--	--	60℃ **(70℃)**[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.

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