AFTMag Quick Tissue/Cell RNA Extraction Kit for Animal

Cat. No. : RK30155

ABclonal Technology

Product components

Components	Component number	Size-1 20 RXN	Size-2 100 RXN	Store
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	RM30293	80 μL	2 × 220 μL	-20°C
DNase I Buffer	RM30294	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 should be stored at -20°C.

DNase I Buffer should be stored at 4°C.

Other reagents can be stored and transported 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.

E-mail:info@abclonal.com

For research purposes only. Not for therapeutic or diagnostic purposes.
Please visit www.abclonal.com for a complete listing of recommended products.

(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. Self-prepared reagent: Proteinase K (When extracting RNA from tissues with high protein content such as heart, muscle, brain and adipose tissue, Proteinase K needs to be added after lysis).

Product Performance Indicators

1. The average yield of 293F cell sample extraction is shown in the table below:

Amount of cells	1000	2000	5000	1×10^4	1×10^5	1×10^6	2×10^6	5×10^6	1×10^7
RNA yield (μg)	0.1	0.16	0.27	0.64	5	30	45	112	183

The sample yield of different tissues extracted from mice is shown in the table below:

Tissue	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: The value of A260/A280 is above 1.8; the value of A260/A230 is generally above 1.7. When extracting trace cells (10^3-10^4 cells), the value of A260/A230 is above 1.0.
3. Extracted nucleic acid integrity: Running intact total RNA on a denaturing gel should produce clear 28S and 18S rRNA bands with a 2:1 ratio (28S:18S), indicating intact RNA. Or the RQN value of capillary electrophoresis is greater than 8.

Operating Instructions

Experimental preparation

Depending on the number of samples, prepare the appropriate volume of DNase I reaction mixture (the dosage for each reaction is shown in Table 1) and dispense it into 96-well plates at the appropriate station or put it on ice/4°C in the refrigerator for use (manual extraction).The DNase I reaction mix should be used as much as possible as it is prepared now to avoid the decrease of enzyme activity.

Table 1. DNase I reaction mixture

Components	Volume per reaction
DNase I	4 μL
DNase I Buffer	96 μL

Note: Before using DNase I, centrifuge briefly to centrifuge the liquid on the tube wall to the bottom of the tube.When the number of cells <10^5 or tissue with low DNA content is extracted, the amount of DNase I can be reduced to 2-3 μL, and the amount of DNase I Buffer can be adjusted to 98 μL-97 μL accordingly.

Sample lysis and homogenization

1. **Suspension cells**

Take an appropriate amount of fresh cell suspension (the number of cells is recommended to be no more than 10^7) into a 1.5 mL centrifuge tube, centrifuge at 600 × g for 5 min at 4°C, discard the supernatant and add the recommended amount of Mag Buffer RLA (the amount of which is referred to in Table 2), and repeatedly pipette to resuspend and lyse the cells or vortex to lyse the cells until the homogenate is clear and transparent.

E-mail:info@abclonal.com

For research purposes only. Not for therapeutic or diagnostic purposes.
Please visit www.abclonal.com for a complete listing of recommended products.

2. Adherent cells

For monolayer adherent cells, discard the supernatant of the culture medium. Use 1X PBS to wash away the residual culture medium to avoid affecting downstream reactions. After that, the lysis solution can be directly added to the culture dish for lysis.

(1) Adherent cells with a quantity of $\leq 10^7$: add the recommended amount of lysis solution Mag Buffer RLA (the amount of which is referred to in Table 2) and pipette repeatedly to make the cells detach and lyse.

(2) Adherent cells with a quantity of $>10^7$: Digest with trypsin and resuspend with PBS. Take a certain number of cell suspensions and lyse according to the method for suspension cells.

(3) Adherent cells in 96-well plates: After discarding the culture medium or PBS washing solution, add 110 μL/well of freshly prepared lysis mixture (prepared according to 100 μL Mag Buffer RLA + 10 μL proteinase K (20 mg/mL) per well. Prepare for immediate use. The prepared lysis mixture needs to be added to the cells within 30 minutes). Pipette repeatedly to make the cells detach and lyse.

Table 2. Recommended dosage of Mag Buffer RLA for different cell inputs

Amount of cells	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

Take 500 μL of Trizol-preserved cell homogenate, add 200 μL of Mag Buffer RLA, and mix well, and then added into 300 μL of Mag Buffer BD for subsequent extraction.

Note: For freshly cultured cells, generally 10^5 -10^7 cells are added with 1 mL of Trizol, mixed and immediately frozen at -80℃ for use.

For freshly cultured cells, generally add 1 mL of Trizol to the cell pellet of 10^5 - 10^7 cells. After mixing evenly, immediately freeze and store it at -80℃ for later use. When using, take 500 μL of the cell homogenate preserved in Trizol, add 200 μL of Mag Buffer RLA and mix thoroughly. Then add it to 300 μL of Mag Buffer BD for subsequent extraction.

Note: The volume of Mag Buffer BD in the regular pre-packaged reagents is 400 μL. 100 μL needs to be pipetted up and discarded, and 300 μL should be reserved for use.

4. Animal tissue (5-30 mg)

Table 3. Recommended input amounts for different tissues

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

Note: If a certain tissue with no reference range for RNA abundance is extracted for the first time, it is recommended that the sample size be tested starting at 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 (ensuring multiple short grinding times avoids degradation of the samples as they warm up).

E-mail:info@abclonal.com

For research purposes only. Not for therapeutic or diagnostic purposes.
Please visit www.abclonal.com for a complete listing of recommended products.

b. Add 500 μL of **Mag Buffer RLA** (If the temperature is too low, the liquid will freeze. You can shake the centrifuge tube to make it melt quickly), vortex and mix for 2 min until there is no obvious lumpy/flocculent tissue particles in the tube. (For tissues with high protein content such as heart and muscle, as well as fat and brain tissue, 0.2 mg of proteinase K can be added per sample after lysis.. After thorough mixing, let it stand for 2 to 3 minutes.) Centrifuge at 16,000 × g for 5 min at 4°C, and take the supernatant for subsequent extraction.

(2)Wet grinding

c. 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 of **Mag Buffer RLA** and three 5 mm steel beads, and grind at 60 Hz for 30 s.(For tissues with high protein content such as heart and muscle, as well as fat and brain tissue, 0.2 mg of proteinase K can be added per sample after lysis. After thorough mixing, let it stand for 2 to 3 minutes.) Centrifuge at 16,000 × g for 5 min at 4°C, and take the supernatant for subsequent extraction.

(3)Preservation Solution Preserved Tissue

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 Sections of Tissue (≤10 slices, slice thickness 4-20 μm)**

Take frozen tissue sections into a liquid nitrogen pre-cooled centrifuge tube, immediately add 500 μL of **Mag Buffer RLA**, quickly vortex and shake or blow with a pipette to mix until there is no obvious flocculent precipitate. Centrifuge at 16,000 × g for 5 min at 4°C, and take the supernatant for subsequent extraction.

Note: For tissues without reference range, it is recommended that the thickness of the initial extraction slice is 10 μm, and the number of slices is 2, and then adjusted according to the RNA extraction rate.

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. 96-well plate reagents dispensing chart

Components	Volume per well	16 RXN - Column	96 RXN - Station
Mag Buffer BD	400 μL/well	Columns 1/7	Station 1
Mag Buffer WA1	800 μL/well	Columns 2/8	Station 2
Mag Buffer WA2 + Mag Beads A	800 μL+10 μL/well	Columns 3/9	Station 3
DNase I Reaction Mixture	100 μL/well	Columns 4/10	Station 4
Mag Buffer WA3	800 μL/well	Columns 6/12	Station 5
RNase-free Water	30-100 μL/well	Columns 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 concentrate the reagent and magnetic beads at the bottom of the plate (a plate centrifuge can also be used and centrifuged at 500 × rpm for 1 minute). Carefully tear off the sealing film (avoid plate vibration and liquid splashing). Add the prepared **DNase I reaction mixture** to Columns 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 Columns 1/7 of the 96-well plate (S-16/S-48 extractor) or the 96-well plate corresponding to Plate 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,

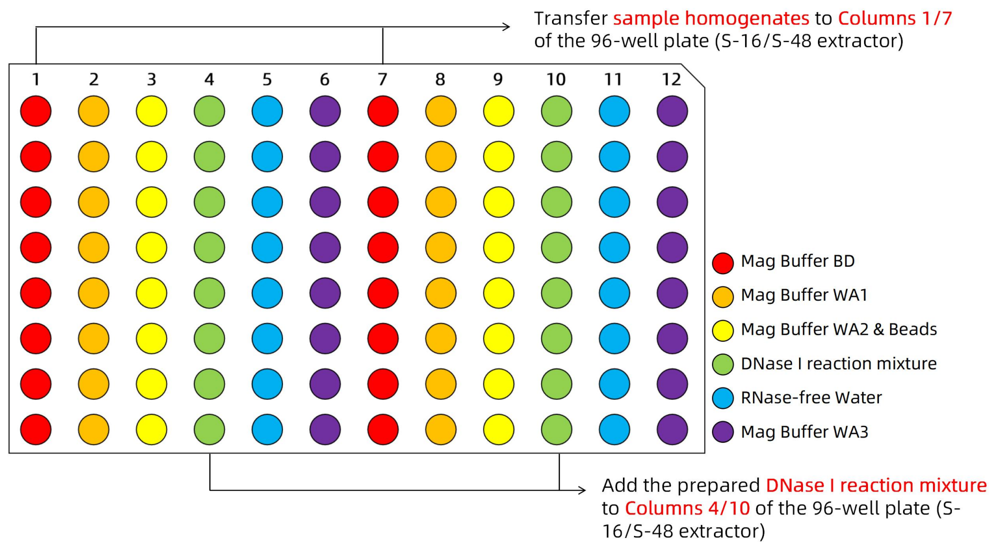
E-mail:info@abclonal.com

For research purposes only. Not for therapeutic or diagnostic purposes.
Please visit www.abclonal.com for a complete listing of recommended products.

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 the Columns 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.

Schematic Diagram of Reagent dispensing



Extraction protocols for each model of instrument

Table 5. Automated extraction protocol on S-16/S-48 device

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	M2	M1	M2	M1	M1
Mix Time	00:00:00	00:05:00	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	800 μL	810 μL	100 μL	800 μL	100 μL	810 μL
Temperature	--	25℃	--	--	--	--	60℃	--

Table 6. Automated extraction protocol on N-96 device

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	M2	M1	M2	M1	M1
Mix Time	00:00:00	00:05:00	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	800 μL	810 μL	100 μL	800 μL	100 μL	810 μL
Temperature	--	25℃	--	--	--	--	60℃	--

E-mail:info@abclonal.com

For research purposes only. Not for therapeutic or diagnostic purposes.
Please visit www.abclonal.com for a complete listing of recommended products.

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 seconds (to avoid liquid residue on the tube cap) and then placed on a magnetic stand. Let it stand for 10 seconds. 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 minutes. Briefly centrifuge the centrifuge tube for 5 seconds and then place it on the magnetic stand. Let it stand for 10 seconds. 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 minutes. Briefly centrifuge the centrifuge tube for 5 seconds and then place it on the magnetic stand. Let it stand for 10 seconds. 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 minutes.
6. Add 100 μL of prepared **DNase I reaction mixture** directly into the centrifuge tube, pipette and mix to resuspend the magnetic beads, and place it at room temperature for 15 minutes (during this period, use your finger to flick the bottom of the centrifuge tube every 5 minutes to fully mix the magnetic beads and the reaction solution). After digestion is completed, briefly centrifuge the centrifuge tube for 5 seconds and then place it on the magnetic stand. Let it stand for 10 seconds. 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 minutes. Briefly centrifuge the centrifuge tube for 5 seconds and then place it on the magnetic stand. Let it stand for 10 seconds. 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 minutes. 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°C for 5 minutes (Or heat for 5 minutes, 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 seconds. 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: To avoid the influence of residual liquid from each operation step on the next washing or elution, the centrifuge tube can be briefly centrifuged for 5 seconds before being placed on the magnetic stand to remove the residual liquid on the centrifuge tube cap.

E-mail:info@abclonal.com

For research purposes only. Not for therapeutic or diagnostic purposes.
Please visit www.abclonal.com for a complete listing of recommended products.