AFTMag Quick Complex Plant RNA Extraction Kit

Cat. No. : RK30180

Product components

Components	Component number	Size-1 20 RXN	Size-2 100 RXN	Storage
Mag Buffer RLM	RM30478	15 mL	70 mL	RT
Mag Buffer BDH	RM30479	10 mL	50 mL	RT
Mag Buffer WM1	RM30480	15 mL	75 mL	RT
Mag Buffer WM2 & Beads	RM30481	15 mL	75 mL	RT
Mag Buffer WM2	RM30482	15 mL	75 mL	RT
Mag Buffer EA	RM30311	4 mL	20 mL	RT
DNase I, RNase-free (5,000 U/mL)	RM21312	40 μL	220 μL	-20℃
DNase I Buffer	RM30294	2 × 1.25 mL	12.5 mL	4℃
CY Reagent	RM30325	400 μL	2 × 1 mL	-20℃

Product Description

This kit uses magnetic bead-based technology for nucleic acid purification. It utilizes functional groups on magnetic beads to enrich nucleic acids from sample lysates onto the bead surface.The beads are then washed to remove proteins and other impurities. After that, a magnetic separation device is used to separate the magnetic beads, and then DNase I is used for digestion to remove DNA, so as to rapidly separate and purify RNA. The whole process does not require the use of phenol, chloroform and β-merphytoethanol and other harmful substances, safe and non-toxic, and the use of paramagnetic magnetic beads, suitable for high-throughput automated extraction.

Storage

CY Reagent: Store at -20℃;

DNase I: Store at -20℃;

DNase I Buffer: Store at 4℃;

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

Applicable Samples

Sample Types	Applicable Plant Species
Roots, Stems, Leaves	Cotton, sweet potato, banana, sesame, rape, tomato, green tea, camellia, coffee, spinach, pak choi, coriander, chili, wolfberry, scallion, broccoli, alfalfa, etc.; It can also be compatible with some common plant samples such as rice, wheat, corn, potato, tobacco, soybean, and Arabidopsis thaliana.
Seeds	Rice, corn, wheat, Arabidopsis thaliana, rape, peanut, soybean, mung bean, coffee
Fruit Pulp	Apple, tomato, citrus, cucumber, sweet potato, green bean, snow pea
Peel	Lemon, citrus
Fungi	Tremella aurantialba, Tremella fuciformis

Notes

1. Throughout the process of plant RNA extraction using this kit, all contamination by RNase should be avoided, including but not limited to consumables such as latex gloves used, pipette tips, centrifuge tubes for sample grinding, mortars, etc. Therefore, inferior gloves should be avoided. Gloves need to be changed frequently during the operation, and masks should be worn properly. It is necessary to avoid a large number of people coming and going in the extraction environment and having conversations with others.

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2. It is recommended to use high-quality RNase-free consumables. If there are no reliable consumables available, you can treat the consumables with 0.2% DEPC water overnight by yourself. The treated consumables can be completely free of residual DEPC after being dried in an oven at 65℃ overnight.
3. High-quality RNA extracted from plants should have an A260/A280 ratio between 1.8-2.2 and an A260/A230 ratio between 1.8-2.2 when measured by spectrophotometry. Abnormal A260/A280 ratios suggest protein contamination. Agarose gel electrophoresis can further confirm protein residue. If the agarose gel electrophoresis shows obvious bright sample wells and diffuse smearing, it indicates protein residue.

Operation Description

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℃ refrigerator for later use . The DNase I reaction mix should be prepared fresh to avoid reduced enzyme activity.

Table 1. DNase I Reaction Mix Preparation

Components	Volume per reaction
DNase I, RNase-free (5,000 U/mL)	2 μL
DNase I Buffer	98 μL

Sample Lysis

- Young plant roots, stems, leaves, and etiolated seedlings are suitable for liquid nitrogen grinding and wet grinding.
- Plant seed samples and older roots, stems, and leaves are suitable for liquid nitrogen mortar grinding and liquid nitrogen automated grinding (large amounts of tissue, hard tissue, high water content tissue). Small seeds can be processed with liquid nitrogen automated grinding (small amounts of tissue).
- Plant pulp and peel are suitable for liquid nitrogen mortar grinding and liquid nitrogen automated grinding (large amounts of tissue, hard tissue, high water content tissue).
- Fungal samples such as Tremella fuciformis and Tremella aurantialba are suitable for liquid nitrogen mortar grinding and liquid nitrogen automated grinding (large amounts of tissue, hard tissue, high water content tissue).

1. Liquid Nitrogen Grinding Lysis

(1) Grinding

a. **Mortar**: Place plant samples into a mortar under liquid nitrogen and grind into fine powder.

b. **Grinding Rod**: Place samples in a 1.5 mL tube, submerge in liquid nitrogen, and grind with pre-cooled rod.

c. **Automated Grinding (Small Amount of Tissue)**: Place plant samples in a 2 mL centrifuge tube containing three 3 mm steel beads, tighten the lid, and freeze in liquid nitrogen for 30 s. Simultaneously, freeze the grinder adapter in liquid nitrogen for 30 s. Grind at 60 Hz for 30 s, repeating three times. After each grinding pause, freeze in liquid nitrogen for 30 s before continuing.

d. **Automated Grinding (Large Amounts of Tissue, Hard Tissue, High Water Content Tissue)**: Use a 15/30/50 mL steel jar with a compatible grinder. Pre-cool the steel jar in liquid nitrogen, place the plant sample into the steel jar, tighten it, freeze in liquid nitrogen for 30 s, then place in the grinder. Grind at 60 Hz for 30 s, repeating three times. After each grinding pause, freeze in liquid nitrogen for 30 s before continuing.

Note: Transfer powder to liquid nitrogen-pre-cooled tubes immediately after grinding. Store at -80℃ for long-term storage or keep in liquid nitrogen for immediate extraction.

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(2) Lysis

a. Transfer ground powder to liquid nitrogen-pre-cooled RNase-free tubes. Add **Mag Buffer RLM** and 15-20 μL **CY Reagent** according to the recommendations in Table 2. Vortex thoroughly for 3 min and incubate at room temperature for 10 min for lysis.

b. Centrifuge at 15,000 rpm, 4℃ for 5-10 min. After centrifugation, place the centrifuge tubes on ice. When extraction reagents are ready, transfer 500 μL of supernatant to **Column 1/7 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 1 (N-96/S-96 extractor)** for automated extraction, or transfer to centrifuge tubes for manual extraction.

Table 2. Recommended Lysis Buffer Volumes for Different Sample Inputs

Sample Amount (mg)	Mag Buffer RLM (μL)
25-100	600
100-200	700
200-300	1000
*Seeds 10-25	1000
*Pulp 100-300	600-1000

2. Wet Grinding Lysis (Fresh Samples Only)

a. Place plant samples in a 2 mL centrifuge tube containing three 4-5 mm steel beads. Add **Mag Buffer RLM** and 15-20 μL **CY Reagent** according to Table 2 recommendations. Grind at at 70 Hz for 5 min.

b. Centrifuge at 15,000 rpm, 4 ℃ for 5-10 min. After centrifugation, place the centrifuge tubes on ice. When extraction reagents are ready, transfer 500 μL of supernatant to **Column 1/7 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 1 (N-96/S-96 extractor)** for automated extraction, or transfer to centrifuge tubes for manual extraction.

3. Direct Lysis (Plant Protoplasts)

a. After centrifugation at 100 g in soft mode for 2 min, carefully discard the supernatant. Add 400 μL **Mag Buffer RLM** and 15 μL **CY Reagent**, mix by vortexing for 1-2 min, and incubate at room temperature for 5-10 min for lysis. Use entire lysate for extraction without centrifugation.

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 4-6.
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 3. Reagent Dispensing Volumes and Positions

Components	Volume per well	16 RXN - Column	96 RXN - Station
Mag Buffer BDH	450 μL	Column 1/7	Station 1
Mag Buffer WM1	700 μL	Column 2/8	Station 2
Mag Buffer WM2 & Beads	700 μL	Column 3/9	Station 3
DNase I Reaction Mix	100 μL	Column 6/12	Station 4
Mag Buffer WM2	700 μL	Column 4/10	Station 5
Mag Buffer EA	50-100 μL	Column 5/11	Station 6

3. For pre-packaged reagents, gently flick the plate to collect reagents and beads at the bottom (or centrifuge the plate 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 6/12 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 4**

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(N-96/S-96 extractor).

4. Process samples according to the sample lysis protocol and transfer **500 µL of centrifuged sample supernatant** to **Column 1/7 of the 96-well plate (S-16/S-48 extractor)** or **the 96-well plate corresponding to Station 1 (N-96/S-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/S-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.

Manual Extraction

1. Thoroughly vortex **Mag Buffer WM2 & Beads**. Pipette 700 µL into a 1.5 mL centrifuge tube. Place the tube on a magnetic rack and let stand for 30 s. Transfer all of the supernatant to a new 1.5 mL centrifuge tube for later use (the supernatant is **Mag Buffer WM2**). Retain the beads for later use.
2. Thoroughly resuspend **Mag Buffer BDH**. Pipette 450 µL of **Mag Buffer BDH** into the tube containing the beads from the previous step.
3. Pipette 500 µL of the centrifuged sample supernatant (if the supernatant volume is large, you may pipette up to 1000 µL) into the tube from the previous step containing Mag Buffer BDH and beads. Vortex to mix for 5 min. Place the tube on the magnetic rack and let stand for 30 s. Discard all supernatant.

   Note: The formation of flocculent precipitate is normal; ensure thorough mixing by vortexing.
4. Remove the tube from the magnetic rack. Add 700 µL of **Mag Buffer WM1**. Vortex to mix for 2 min. Place the tube on the magnetic rack and let stand for 30 s. Discard all supernatant.
5. Add the 700 µL of **Mag Buffer WM2** collected in Step 1 to the tube. Vortex to mix for 2 min. Place the tube on the magnetic rack and let stand for 30 s. Discard all supernatant.
6. Air-dry with the lid open for 15 s. Add 100 µL of **DNase I Reaction Mix**. Vortex gently at room temperature for 15 min. Place the tube on the magnetic rack and let stand for 30 s. Discard all supernatant.
7. Remove the tube from the magnetic rack. Add 700 µL of **Mag Buffer WM2**. Vortex to mix for 2 min. Place the tube on the magnetic rack and let stand for 30 s. Discard all supernatant.
8. Air-dry with the lid open for 2 minutes. (During this time, preheat the Elution Buffer **Mag Buffer EA** to 55°C.) After the beads are dry, add 50-100 µL of **Mag Buffer EA**. Incubate at 55°C on a rotating metal bath for 3.5 min to dissolve. Place the tube on the magnetic rack and let stand for 1 min. Carefully transfer all of the liquid to a new RNase-free centrifuge tube, taking care not to pipette any beads. Store short-term at -20°C or long-term at -80°C.

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Extraction Programs for Different Instrument Models

Table 4. S-16/S-48 Automated Nucleic Acid Extractor Reference Program

Step	1	2	3	4	5	6	7	8
Station	3	1	2	3	6	4	5	4
Wait Time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:15	00:00:00	00:02:00	00:00:00
Mix Mode	M3	M1	M3	M3	M1	M3	M2	M3
Mix Time	00:00:10	00:05:00	00:02:00	00:02:00	00:15:00	00:02:00	00:03:30	00:00:20
Pause	No	No	No	No	No	No	No	No
Mag Time	00:00:50	00:01:00	00:00:30	00:00:30	00:00:55	00:00:30	00:01:00	00:00:00
Volume	700 μL	950 μL	700 μL	700 μL	100 μL	700 μL	100 μL	700 μL
Temperature	--	25℃	--	--	--	--	65℃	--

Table 5. S-96 Automated Nucleic Acid Extractor Reference Program

Step	1	2	3	4	5	6	7	8
Plate	3	1	2	3	4	5	6	5
Waiting Time	0:0:0	0:0:0	0:0:0	0:0:0	0:0:15	0:0:0	0:2:0	0:0:0
Mixing Time	0:0:10	0:5:0	0:2:0	0:2:0	0:15:0	0:2:0	0:3:30	0:0:20
Mixing Speed	6	1	6	6	1	6	2	6
Collecting time	0:50	1:0	0:30	0:30	0:55	0:30	1:0	0:0
Lysis: 25℃				Elution: 65℃			Storage: 4℃	

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

Step	1	2	3	4	5	6	7	8
Station	3	1	2	3	4	5	6	5
Wait Time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:15	00:00:00	00:02:00	00:00:00
Mix Mode	6	1	6	6	1	6	2	6
Mix Time	00:00:10	00:05:00	00:02:00	00:02:00	00:15:00	00:02:00	00:03:30	00:00:20
Pause	No	No	No	No	No	No	No	No
Mag Time	00:00:50	00:01:00	00:00:30	00:00:30	00:00:55	00:00:30	00:01:00	00:00:00
Volume	700 μL	950 μL	700 μL	700 μL	100 μL	700 μL	100 μL	700 μL
Temperature	--	25℃	--	--	--	--	65℃	--

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