AFTMag Quick Plant Leaf DNA Extraction Kit

Cat. No. : RK30159

Product components

Components	Component number	Size-1 20 RXN	Size-2 100 RXN	Storage
Mag Buffer RLB	RM30300	13 mL	65 mL	RT
Mag Buffer WC1 & Beads	RM30301	11 mL	55 mL	RT
Mag Buffer WC2	RM30302	22 mL	110 mL	RT
Mag Buffer EB	RM30229	4 mL	20 mL	RT
RNase A (10 mg/mL)	RM30304	100 μL	600 μL	-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, and then separate magnetic beads with magnetic separation device, so as to quickly separate and purify DNA from samples. The whole process does not require the use of phenol, chloroform and β-merphytoethanol,, safe and non-toxic, and the use of paramagnetic magnetic beads, suitable for high-throughput automated extraction.

Storage

RNase A should be stored at -20℃, and other components of the kit should be stored at room temperature (10-30℃).

Applicable samples

Suitable for fresh leaves, dried leaves, and freeze-dried leaves.

Samples extracted within 1 day can be stored at 2-8℃.

Samples extracted for more than 1 week should be stored at -20℃ or -80℃ or dried for preservation.

Precautions

1. Instruments and equipment should be regularly disinfected with UV and cleaned with 75% ethanol.
2. It is normal for Mag Buffer RLB and Mag Buffer WC2 to precipitate. Place them in an oven at 65℃ or a water bath. After the precipitation is dissolved, mix them well before use.
3. The deep well plate or reagent bottle containing magnetic beads must be shaken well before use.
4. Self-prepared reagent: isopropanol.

Automated Extraction

1. Turn on the power of the instrument and wait for it to complete the self-test. Then set the instrument parameters as shown in the following table.

Table 1. Automated extraction protocol on S-96 device

Step	1	2	3	4	5	6	7
Plate	2	1	2	3	4	6	2
Waiting Time	0	0	0	0	0	3 min	0
Mixing Time	0	5 min	2 min	2 min	2 min	3 min	1 min
Mixing Speed	6	6	6	6	6	1	6
Collecting time	1 min	1 min	1 min	1 min	1 min	1 min	0
Lysis: 25℃			Elution: 65℃			Storage: 4℃	

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Table 2. Automated extraction protocol on S-16/S-48 device

Step	1	2	3	4	5	6	7
Station	2	1	2	3	4	5	2
Wait Time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:00	00:03:00	00:00:00
Mix Mode	2	2	2	2	2	1	2
Mix Time	00:00:00	00:05:00	00:02:00	00:02:00	00:02:00	00:03:00	00:00:30
Pause	No	No	No	No	No	No	No
Mag Time	00:01:00	00:01:00	00:01:00	00:01:00	00:01:00	00:01:00	00:00:00
Volume	500 μL	700 μL	500 μL	500 μL	500 μL	100 μL	500 μL
Temperature	--	25℃	--	--	--	65℃	--

Table 3. Automated extraction protocol on N-96 device

Step	1	2	3	4	5	6	7
Station	2	1	2	3	4	6	2
Wait Time	00:00:00	00:00:00	00:00:00	00:00:00	00:00:00	00:03:00	00:00:00
Mix Mode	4	4	4	4	4	2	4
Mix Time	00:01:00	00:05:00	00:02:00	00:02:00	00:02:00	00:03:00	00:00:30
Pause	No	No	No	No	No	No	No
Mag Time	00:01:00	00:01:00	00:01:00	00:01:00	00:01:00	00:01:00	00:00:00
Volume	500 μL	700 μL	500 μL	500 μL	500 μL	100 μL	500 μL
Temperature	--	25℃	--	--	--	65℃	--

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	96 RXN-Station	16 RXN-Column
Sample	/	Station 1	Column 1/7
Mag Buffer WC1 & Beads	500 μL	Station 2	Column 2/8
Mag Buffer WC2	500 μL	Station 3	Column 3/9
Mag Buffer WC2	500 μL	Station 4	Column 4/10
Mag Buffer EB	100 μL	Station 6	Column 5/11

3. Place an appropriate amount of plant tissue (20-100 mg for fresh or freeze-dried samples, 15-30 mg for dried samples) into a 2 mL centrifuge tube. Add one 4 mm (diameter) steel bead to the tube, along with 600 μL of **Mag Buffer RLB** and 5 μL of **RNase A** (10 mg/mL). Use a grinder at 60 Hz to crush the sample, running for 60 s with a 10 s pause, repeating the grinding process 4-6 times. According to the difficulty of sample grinding, the number of grinding cycles can be appropriately increased or decreased (if there is too much foam, centrifuge at a low speed for 5 seconds).
4. After mixing by shaking, place the tube in a metal bath at 70℃ for 8 minutes, mixing 1-2 times.
5. Once lysis is complete, centrifuge the tube at 12,000 × rpm for 8 minutes. Transfer 400 μL of the supernatant to the corresponding wells of the sample plate, and then add 300 μL of isopropanol to each sample well.
6. Place the 96-well plate with the dispensed reagents into the corresponding station of the instrument according to Table 4 (Before using the prepackaged reagents, invert it several times to mix well, then gently shake the plate to concentrate the solution at the bottom of the wells. Carefully remove the sealing film to avoid plate vibration and liquid splashing), and run the program.

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7. After the program ends, the instrument will automatically stop. The extracted nucleic acid samples are contained in the deep-well plate corresponding to the elution buffer Mag Buffer EB (for S-48/S-16 device, the extracted nucleic acid samples are in columns 5 and 11 of the deep-well plate.) These samples can be directly used for downstream experiments. If long-term storage is required, they can be packaged or transferred to new centrifuge tube and stored in a -20°C refrigerator.

Manual Extraction

1. Place an appropriate amount of plant tissue (20 - 100 mg of fresh or freeze-dried sample, 15 - 30 mg of dried sample) into a 2 mL centrifuge tube. Add one 4 mm (diameter) steel ball to the tube, followed by 600 µL of **Mag Buffer RLB** and 5 µL of **RNase A (10 mg/mL)**. Use a grinder at 60 Hz to crush the sample for 60 s with a 10 s pause, repeating the grinding process 4-6 times. Adjust the number of grinding cycles according to the difficulty of sample grinding (if too much foam is generated, centrifuge at low speed for 5 s).
2. After vortex mixing, place the sample in a metal bath at 70°C for lysis for 8 minutes, mixing 1-2 times during the process.
3. After lysis is complete, centrifuge the tube at 12,000 × rpm for 6~8 minutes.
4. During centrifugation, mix the **Mag Buffer WC1 & Beads** thoroughly and transfer 500 µL into a new centrifuge tube (labeled as ①). Place the tube on a magnetic stand for 1 minute, then transfer all the supernatant into another new centrifuge tube for later use (labeled as ②, which is Mag Buffer WC1). Preserve the magnetic beads for subsequent use.
5. After the centrifugation in Step 3 above is completed, take 400 µL of the supernatant and transfer it to the centrifuge tube containing magnetic beads (i.e., the tube labeled as ① above). Then, add 300 µL of isopropanol to the centrifuge tube and mix it on a mixer for 5-10 minutes (or mix it by shaking and let it stand for 5 - 10 minutes, during which shake and mix it 3-5 times).
6. Place the centrifuge tube on a magnetic stand and let stand for 1 minute. During this time, invert the magnetic stand 5~6 times to ensure that any residual magnetic beads on the tube cap are adsorbed. Carefully pipette out and discard all the liquid from the tube cap and tube.
7. Remove the centrifuge tube and add 500 µL of **Mag Buffer WC1** (i.e., the liquid from the tube labeled as ② that was saved for later use). Shake the tube to mix for 1 minute, then place it back on the magnetic stand and let stand for 1 minute. During this time, invert the magnetic stand 5~6 times. After the magnetic beads are completely adsorbed, carefully pipette out the liquid from the tube cap and tube.
8. Add 500 µL of **Mag Buffer WC2** and repeat the process in step 7.
9. Repeat step 8.
10. Open the tube cap and dry it at room temperature for 5 minutes until the ethanol evaporates completely (both residual ethanol and excessive drying will affect the efficiency of nucleic acid elution in the next step).
11. Remove the centrifuge tube, add 100 µL of **Mag Buffer EB**, shake and mix well to ensure that the magnetic beads are completely immersed in the elution buffer, and then incubate at 65°C for 5 minutes, shaking and mixing evenly 3 times during this period.
12. After incubation, place the centrifuge tube on a magnetic stand and let stand for 1 minute. Once the magnetic beads are completely adsorbed, carefully pipette the liquid into a new, clean centrifuge tube (avoid pipetting out the magnetic beads). The resulting solution is the nucleic acid sample. This sample can be used directly for downstream experiments or stored at -20°C for long-term preservation.

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