AFTMag Stool/Soil DNA Extraction Kit

Cat. No. : RK30157

ABclonal Technology

Product components

Components	Component number	Size-1 20 RXN	Size-2 100 RXN	Storage
Mag Buffer RA	RM30266	9 mL	55 mL	RT
Mag Buffer RB	RM30265	2 mL	12 mL	RT
Mag Buffer RC	RM30267	2 mL	12 mL	RT
Mag Buffer RL2	RM30264	13 mL	65 mL	RT
Mag Buffer WG1 & Beads	RM30268	13 mL	65 mL	RT
Mag Buffer WG2	RM30269	26 mL	2 × 65 mL	RT
Mag Buffer EB	RM30229	4 mL	20 mL	RT
RNase A (10 mg/mL)	RM30304	200 μL	1.2 mL	-20℃
Extraction Tube (Zirconia Beads)	RM30554	20 pk	5 × 20 pk	RT

Product Description

This kit is a nucleic acid extraction kit based on bio-nanomagnetic beads. The main principle involves using 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 beads are then washed to remove proteins and other impurities. Then, a magnetic separation device is used to isolate the beads, allowing for rapid separation and purification of nucleic acids. The entire process does not require the use of organic reagents such as phenol, chloroform, or β-mercaptoethanol, making it safe and non-toxic. The use of paramagnetic magnetic beads is suitable for high-throughput automated extraction. The extracted product can be directly used for RT-PCR, RT-qPCR, Northern Blot, next-generation sequencing library construction, and cloning.

Storage

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

Applicable samples

It is applicable to the extraction of genomic DNA from 100 - 500 mg soil and environmental samples (such as wastewater, feces, sludge, garbage, gypsum, food, fungi, plants, roots, yeast, sediment, etc.).

Precautions

1. Extraction should be conducted in designated experimental areas with protective clothing, disposable gloves, and masks.
2. Unless otherwise specified, all operations should be completed at room temperature (10 - 30℃).
3. The fully automatic nucleic acid extractor should be disinfected with ultraviolet rays before the experiment, and the laboratory bench and the pipette dedicated for sample extraction should be cleaned regularly with 75% ethanol.
4. Precipitates may precipitate out in Mag Buffer RA, Mag Buffer RB and Mag Buffer RL2, which is a normal phenomenon. Place them in a 42℃ oven or water bath, and mix them evenly after the precipitates are dissolved before using.
5. During the manual extraction and cleaning process, the cleaning solution at each step must be sucked clean. If there are liquid droplets on the tube wall, you can tap the magnetic rack gently to let the droplets flow to the bottom of the preparation tube and then suck them clean.
6. During manual extraction, the magnetic beads should not be left to dry for too long. Excessive drying will affect the extraction results.

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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 DNA 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	2 min	0
Mixing Time	30 s	7 min	4 min	1 min	1 min	3 min	30 s
Mixing Speed	6	6	6	6	6	6	6
Collecting time	1 min	1 min 30 s	1 min	1 min	1 min	1 min 30 s	0
	Lysis: 25℃			Elution: 65℃			Storage: 4℃

Table 2. Automated DNA 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	00:02:00	00:00:00
Mix Mode	4	4	4	4	4	3	4
Mix Time	00:00:30	00:07:00	00:04:00	00:01:00	00:01:00	00:03:00	00:00:30
Pause	No	No	No	No	No	No	No
Mag Time	00:01:00	00:01:30	00:01:00	00:01:00	00:01:00	00:01:30	00:00:00
Volume	600 μL	900 μL	600 μL	600 μL	600 μL	100 μL	600 μL
Temperature	--	25℃	--	--	--	65℃	--

Table 3. Automated DNA 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	00:02:00	00:00:00
Mix Mode	4	4	4	4	4	3	4
Mix Time	00:00:30	00:07:00	00:04:00	00:01:00	00:01:00	00:03:00	00:00:30
Pause	No	No	No	No	No	No	No
Mag Time	00:01:00	00:01:30	00:01:00	00:01:00	00:01:00	00:01:30	00:00:00
Volume	600 μL	900 μL	600 μL	600 μL	600 μL	100 μL	600 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-Plate	16 RXN-Column
Mag Buffer RL2	600 uL	Plate 1	Columns 1/7
Mag Buffer WG1 & Beads	600 uL	Plate 2	Columns 2/8
Mag Buffer WG2	600 uL	Plate 3	Columns 3/9
Mag Buffer WG2	600 uL	Plate 4	Columns 4/10
Mag Buffer EB	100 uL	Plate 6	Columns 5/11

3. Add 400 μL of **Mag Buffer RA**, 60 μL of **Mag Buffer RB** and 10 μL of **RNase A** into the Extraction Tube.

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Note: The volume of Mag Buffer RA can be adjusted appropriately. For fecal samples in the preservation solution, it can be reduced to 300 μL; for solid feces or dry soil, it can be increased to 500 μL.

4. Sample pretreatment:
   (1) Bacterial and fungal culture solutions: Take 100-200 μL of the microbial culture solution and add it to the Extraction Tube; for 200-2000 μL samples, transfer the samples to a centrifuge tube, centrifuge at 12,000 rpm for 2 minutes, discard most of the supernatant, and retain less than 200 μL of liquid to resuspend the microorganisms.
   (2) Water filtration membrane: Roll up the filtration membrane, cut it into several sections, and add them into the Extraction Tube.
   (3) Soil and environmental samples: Add them directly into the Extraction Tube.
   Note: The volume of the sample added into the Extraction Tube should not exceed two-thirds of the height of the Extraction Tube (approximately below the anti-slip ring).
5. Vortex to mix evenly. Use a grinding machine to grind at 60 Hz for 30 seconds, wait for 10 seconds, and repeat this operation 3 times.
   Note: For samples that are relatively difficult to break, the number of grinding times can be appropriately increased to 4-6 times; if there is no grinding machine, vortex on a vortex mixer for 15 minutes.
6. After grinding, transfer the Extraction Tube to a metal bath or water bath and incubate at 70°C for 10 minutes.
   Note: For extremely difficult-to-process samples, the temperature can be increased to 90°C.
7. Take out the Extraction Tube, centrifuge instantaneously for 5-10 seconds, add 80 μL of **Mag Buffer RC**, vortex for 30 seconds to mix evenly, centrifuge at 12,000 rpm for 4 minutes, and transfer the supernatant (about 300 μL) to the wells containing Mag Buffer RL2 (for S-48/S-16 instruments, transfer the supernatant to Columns 1/7 of the deep-well plate). When pipetting, try to avoid aspirating the precipitate as much as possible.
8. Place the 96-well plate with the dispensed reagents into the corresponding position 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.
9. After the program ends, the instrument will stop automatically. The extracted nucleic acid samples are in the deep-well plate corresponding to the elution buffer Mag Buffer EB (for S-48/S-16 instruments, the extracted nucleic acid samples are in the Columns 5/11 of the deep-well plate). This sample can be directly used in 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. Add 400 μL of **Mag Buffer RA**, 60 μL of **Mag Buffer RB** and 10 μL of **RNase A** into the Extraction Tube.
   Note: The volume of Mag Buffer RA can be adjusted appropriately. For fecal samples in the preservation solution, it can be reduced to 300 μL; for solid feces or dry soil, it can be increased to 500 μL.
2. Sample pretreatment:
   (1) Bacterial and fungal culture solutions: Take 100-200 μL of the microbial culture solution and add it to the Extraction Tube; for 200-2000 μL samples, transfer the samples to a centrifuge tube, centrifuge at 12,000 rpm for 2 minutes, discard most of the supernatant, and retain less than 200 μL of liquid to resuspend the microorganisms.
   (2) Water filtration membrane: Roll up the filtration membrane, cut it into several sections, and add them into the Extraction Tube.
   (3) Soil and environmental samples: Add them directly into the Extraction Tube.
   Note: The volume of the sample added into the Extraction Tube should not exceed two-thirds of the height of the Extraction Tube (approximately below the anti-slip ring).

3. Vortex to mix evenly. Use a grinding machine to grind at 60 Hz for 30 seconds, wait for 10 seconds, and repeat this operation 3 times.

   Note: For samples that are relatively difficult to break, the number of grinding times can be appropriately increased to 4-6 times; if there is no grinding machine, vortex on a vortex mixer for 15 minutes.

4. After grinding, transfer the Extraction Tube to a metal bath or water bath and incubate at 70°C for 10 minutes.

   Note: For extremely difficult - to - process samples, the temperature can be increased to 90°C.

5. Take out the Extraction Tube, centrifuge instantaneously for 5-10 seconds, add 80 μL of **Mag Buffer RC**, vortex for 30 seconds to mix evenly, and centrifuge at 12,000 rpm for 4 minutes. Then transfer the supernatant (about 300 μL) to a clean 1.5 mL centrifuge tube.
6. Transfer 600 μL of **Mag Buffer WG1 & Beads** to another clean 1.5 mL centrifuge tube (vortex and mix Mag Buffer WG1 & Beads before transfer). Place the centrifuge tube on a magnetic stand for magnetic adsorption for 1 minute. Transfer the supernatant to another clean 1.5 mL centrifuge tube for standby. Transfer the liquid in step 5 to the centrifuge tube with the remaining magnetic beads.
7. Add 600 μL of **Mag Buffer RL2** and vortex at room temperature for 10 minutes for binding.
8. Place the centrifuge tube on a magnetic stand for magnetic adsorption for 1 minute, discard the supernatant, add the standby liquid in step 6, vortex and mix for 5 minutes, and then place it on the magnetic stand. Let it stand for 1 minute. During this period, invert the magnetic stand 5-6 times repeatedly. After the magnetic beads are completely adsorbed, carefully use a pipette to remove the liquid in the tube cap and the tube.
9. Remove the centrifuge tube, add 600 μL of **Mag Buffer WG2**, shake and mix the centrifuge tube for 2 minutes, and then place it on the magnetic stand. Let it stand for 1 minute. During this period, invert the magnetic stand 5-6 times repeatedly. After the magnetic beads are completely adsorbed, carefully use a pipette to remove the liquid in the tube cap and the tube.
10. Repeat step 9.
11. Open the tube cap and dry at room temperature for 3-5 minutes until the ethanol is completely evaporated (both ethanol residue and excessive drying will affect the nucleic acid elution efficiency in the next step).
12. Remove the centrifuge tube, add 100 μL of the elution buffer **Mag Buffer EB**, shake and mix to make the magnetic beads completely immersed in the elution buffer, and then incubate at 65°C for 5 minutes. During this period, shake and mix 3 times.
13. After incubation, place the centrifuge tube on a magnetic stand and let it stand for 1 minute. After the magnetic beads are completely adsorbed, carefully use a pipette to transfer the liquid to a new clean centrifuge tube (do not aspirate the magnetic beads). The obtained solution is the nucleic acid sample.This sample can be directly used in 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.