7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com
Email: info@abpbio.com

EasyMag DNA Purification Kit from stool

Catalog Number: D124-1, D124-2

Table 1. Kit Components and Storage

Kit Component	D124-1 (50 preps)	D124-2 (200 preps)	Storage	Stability
Buffer ASL	50 mL	2×100 mL	RT	The product is stable for one year when stored as directed.
Buffer DL	25 mL	100 mL	RT	
Buffer AW1*	15 mL	53 mL	RT	
Buffer AW2*	15 mL	2×25 mL	RT	
Buffer AE	15 mL	30 mL	RT	
Proteinase K (20 mg/mL)	1 mL	4×1 mL	-20℃	
MagBinding Beads	2.5 mL	10 mL	2-8 °C	

***** Prior to use, add absolute ethanol to **Buffer AW1, Buffer AW2** according to the bottle label.

Product Description

EasyMag DNA Purification Kit from stool provides fast and easy purification of total DNA from fresh or frozen stool samples. Up to 200 mg of stool samples can be processed in less than 30 minutes. The specially formulated lysis buffer allows direct cell lysis and removes DNA-degrading substances and PCR inhibitors present in the stool sample by centrifugation. The DNA in the supernatant is then purified using magnetic bead technology. Purification requires no phenol or chloroform extraction or alcohol precipitation, and involves minimal handling. The purified DNA is of high quality and well suited for most downstream applications such as PCR, sequencing, genotyping, southern blot analysis and restriction enzyme digestion.

Features

- ❖ Fast – DNA purification process in less than 30 min.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Things to do before starting

- Buffer ASL, Buffer DL may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 70°C.
- Prepare RNase A solution (10 mg/mL).

Protocol: Isolation of DNA from Stool for Human DNA Analysis

1. Weigh ~200 mg stool in a 2 mL microcentrifuge tube and place tube on ice. If the sample is liquid, pipet 200 µL into the microcentrifuge tube. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 mL microcentrifuge tube on ice.
2. Add 1 mL Buffer ASL to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.
3. Centrifuge sample at 13,000 x g for 5 min at RT to pellet stool particles.
4. Add 20 µL Proteinase K solution into a new 2 mL microcentrifuge tube.
5. Transfer 500 µL supernatant from step 3 into the 2 mL microcentrifuge tube containing Proteinase K.

   Note: Do not transfer any solid material.
6. Add 500 µL Buffer DL. Mix by vortexing, and incubate at 70°C for 10 min.

   Note: It is important that the sample and Buffer DL are thoroughly mixed to form a homogeneous solution. Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
7. (*Optional*) If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL). Mix by vortexing, and incubate at RT for 10 min.
8. Add 500 µL isopropanol and 50 µL MagBinding Beads to the lysate, and mix thoroughly by vortexing.
9. Incubate with shaking at RT for 5 min.
10. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
11. Add 600 µL of Buffer AW1 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
12. Add 600 µL of Buffer AW2 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
13. Repeat step 12 for a second wash step.
14. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
15. Add 100 µL Buffer AE to the tube, resuspend the beads by vortexing. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
16. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
17. Store the DNA at -20ºC.

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Protocol: Isolation of DNA from Stool for Pathogen Detection

1. Weigh ~200 mg stool in a 2 mL microcentrifuge tube and place tube on ice. If the sample is liquid, pipet 200 µL into the microcentrifuge tube. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 mL microcentrifuge tube on ice.
2. Add 1 mL Buffer ASL to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.

   Note: It is important to vortex the samples thoroughly. This helps ensure maximum DNA concentration in the final eluate.
3. Heat the suspension for 10 min at 70°C. Vortex for 15 s.

   Note: This heating step helps to lyse bacteria and other parasites. The lysis temperature can be increased to 90°C for cells that are difficult to lyse (such as Gram-positive bacteria).
4. Centrifuge sample at 13,000 x g for 5 min at RT to pellet stool particles.
5. Add 20 µL Proteinase K solution into a new 2 mL microcentrifuge tube.
6. Transfer 500 µL supernatant from step 4 into the 2 mL microcentrifuge tube containing Proteinase K.

   Note: Do not transfer any solid material.
7. Add 500 µL Buffer DL. Mix by vortexing, and incubate at 70°C for 10 min.

   Note: It is important that the sample and Buffer DL are thoroughly mixed to form a homogeneous solution. Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
8. (*Optional*) If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL). Mix by vortexing, and incubate at RT for 10 min.
9. Add 500 µL isopropanol and 50 µL MagBinding Beads to the lysate, and mix thoroughly by vortexing.
10. Incubate with shaking at RT for 5 min.
11. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
12. Add 600 µL of Buffer AW1 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
13. Add 600 µL of Buffer AW2 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
14. Repeat step 13 for a second wash step.
15. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
16. Add 100 µL Buffer AE to the tube, resuspend the beads by vortexing. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
17. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
18. Store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Low yield	• Insufficient homogenization of stool sample in Buffer ASL: Repeat the DNA purification process with a new sample. Be sure to mix the sample and Buffer ASL until the sample is thoroughly homogenized. • Insufficient mixing with Buffer DL: Be sure to mix the sample and Buffer DL immediately and thoroughly by pulse-vortexing. • Improper washing: Buffer AW1, buffer AW2 must be diluted with absolute ethanol before use. • Incomplete resuspension of MagBinding Beads: Resuspend MagBinding Beads by vortexing virgously before use. • DNA remains bound to MagBinding Beads: Increase elution volume and incubation time. • Loss of MagBinding Beads during operation: Avoid disturbing MagBinding Beads during aspiration.
A260/A280 ratio is low	• Insufficient mixing with Buffer DL: Be sure to mix the sample and Buffer DL immediately and thoroughly by pulse-vortexing. • Decreased proteinase activity: Repeat the DNA purification procedure with a new sample and proteinase K.
Poor performance in downstream applications	• Salt contamination: Remove any residual liquid with a pipettor after each wash step. • Ethanol contamination: Ensure the beads are completely dried before elution.