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EasyMag Universal DNA Purification Kit

Catalog Number: D136-1, D136-2

Table 1. Kit Components and Storage

Kit Component	D136-1 (50 preps)	D136-2 (200 preps)	Storage	Stability
Buffer ATL	15 mL	60 mL	RT	The product is stable for one year when stored as directed.
Buffer DL	15 mL	60 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 Universal DNA Purification Kit is designed for rapid purification of genomic DNA from a variety of sample sources including fresh or frozen animal tissues and cells, blood, serum, plasma, saliva, swab, sperm, blood spot. The DNA purification process is simplified with magnetic bead technology into four quick “lyse-bind-wash-elute” steps and can be accomplished in less than 20 minutes post-lysis. This kit can be processed manually or on an automated platform. Purification requires no phenol or chloroform extraction or alcohol precipitation, and involves minimal handling. DNA purified using this kit is ready for most downstream applications such as PCR, sequencing, genotyping, southern blot analysis and restriction enzyme digestion.

Features

- ❖ Fast – DNA purification process in less than 20 min post-lysis.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Things to do before starting

- 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 55°C and 70°C.
- Prepare RNase A solution (10 mg/mL).
- Prepare 1 M DTT solution.

Protocol: Isolation Genomic DNA from Tissue Sample

1. Weigh ~25 mg tissue (or <10 mg liver, lung, or spleen tissue), and cut the tissue into small pieces, then transfer it into a 1.5 mL microcentrifuge tube.

   Note: Using too much tissue sample may reduce yield and purity. Spleen, liver, and kidney tissues are rich in DNA, and should be used no more than 10 mg. Muscle and skin tissues can be used at 30 mg. Liquid nitrogen grinding, mechanical homogenizer, or glass homogenizer can be used to homogenize tissue sample to reduce digestion time.

2. Add 230 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly.

3. Incubate at 55°C for 1-3 hr or overnight. Vortex briefly every 20-30 min during incubation, or incubate with shaking.

   Note: Lysis time depends on the amount and type of tissue used. The average time is 0.5-3 hr, and mouse tails may take 6-8 hr. Lysis can be proceed overnight.

4. (*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.

5. Centrifuge at 12,000 x g for 3 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

6. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve. A precipitate may form upon the addition of Buffer DL. This precipitate does not interfere with the DNA recovery.

7. Add 250 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.

   Note: A precipitate may form upon the addition of isopropanol when processing liver or spleen tissue. Pipette up and down 5-10 times to break up the sediment.

8. Incubate with shaking at RT for 5 min.

9. 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.

10. 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.

11. 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.

12. Repeat step 11 for a second wash step.

13. 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.

14. 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.

15. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.

16. Store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Blood Sample

1. Add 20 µL Proteinase K solution into a 1.5 mL microcentrifuge tube.
2. Transfer 250 µL fresh or frozen anticoagulated whole blood to the 1.5 mL microcentrifuge tube containing Proteinase K. Vortex for 5 seconds.

   Note: When processing coagulated blood samples, homogenize the samples with a mechanical or glass homogenizer to fully liquefy them before extracting. Since red blood cells of non-mammalian animals such as birds and fish are nucleated, their DNA content is extremely rich, and the kit can only process 5-20 µL of blood at a time.
3. Add 250 µL Buffer DL to the sample. Vortex thoroughly for 15 seconds, and then incubate at 70°C for 10 min. Vortex briefly once during incubation.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
4. (*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.
5. Add 250 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.

   Note: A precipitate may form upon the addition of isopropanol when processing liver or spleen tissue. Pipette up and down 5-10 times to break up the sediment.
6. Incubate with shaking at RT for 5 min.
7. 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.
8. 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.
9. 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.
10. Repeat step 9 for a second wash step.
11. 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.
12. 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.
13. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
14. Store the DNA at -20ºC.

Protocol: Isolation Genomic DNA from Serum, Plasma, or Saliva Sample

1. Add 20 µL Proteinase K solution into a 2 mL microcentrifuge tube.
2. Transfer 500 µL serum, plasma, or saliva sample to the 2 mL microcentrifuge tube containing Proteinase K. Vortex for 5 seconds.
3. Add 500 µL Buffer DL to the sample. Vortex thoroughly for 15 seconds, and then incubate at 70°C for 10 min. Vortex briefly once during incubation.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
4. (*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.
5. Add 500 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.
6. Incubate with shaking at RT for 5 min.
7. 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.
8. 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.
9. 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.
10. Repeat step 9 for a second wash step.
11. 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.
12. Add 30-100 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.
13. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
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Protocol: Isolation Genomic DNA from Cultured Cells

1. Prepare the cell suspension using one of the following methods:
   - Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 230 µL PBS. Proceed to Step 2.
   - For cells grown in suspension, pellet 5 x 10^6 by spinning at 1,200 x g in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 230 µL PBS. Proceed to Step 2.
   - For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 230 µL PBS. Proceed to Step 2.
2. Add 20 µL Proteinase K solution. Vortex for 15 seconds. If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL).
3. Add 250 µL Buffer DL to the sample. Vortex thoroughly for 15 seconds, and then incubate at 65°C for 15 min. Vortex briefly once during incubation.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50°C to dissolve.
4. Add 250 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.
5. Incubate with shaking at RT for 5 min.
6. 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.
7. 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.
8. 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.
9. Repeat step 8 for a second wash step.
10. 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.
11. Add 30-100 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.
12. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
13. Store the DNA at -20ºC.

Protocol: Isolation Genomic DNA from Swab Sample

1. Place swab sample in a 2 mL microcentrifuge tube.
2. Add 500 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly.
3. Incubate at 55°C for 1 hr. Vortex briefly every 10 min during incubation, or incubate with shaking.
4. Remove the swab by pressing it against the walls of the tube to squeeze out most of the liquid. Then, centrifuge at 12,000 x g for 3 min. Transfer the supernatant to a new 2 mL microcentrifuge tube.
5. Add 500 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
6. Add 500 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.
7. Incubate with shaking at RT for 5 min.
8. 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.
9. 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.
10. 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.
11. Repeat step 10 for a second wash step.
12. 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.
13. Add 30-100 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.
14. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
15. Store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Blood or Sperm Spot

1. Use a punch to cut out 3-5 blood-bearing discs with a diameter of ~3 mm from the dried blood slices. Transfer it to a 2 mL microcentrifuge tube.
2. Add 230 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly. When processing sperm spot sample, add additional 10 µL of 1 M DTT.
3. Incubate at 55°C for 30-60 min. Vortex briefly every 10 min during incubation, or incubate with shaking.
4. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 15 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
5. Centrifuge at 10,000 x g for 1 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
6. Add 250 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.
7. Incubate with shaking at RT for 5 min.
8. 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.
9. 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.
10. 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.
11. Repeat step 10 for a second wash step.
12. 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.
13. Add 30-100 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.
14. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
15. Store the DNA at -20ºC.

Protocol: Isolation Genomic DNA from Hair or Nail Sample

1. Cut hair or nail sample into small pieces, and transfer it into a 1.5 mL microcentrifuge tube.
2. Add 230 µL Buffer ATL, 20 µL Proteinase K solution, and 10 µL DTT (1 M). Vortex to mix thoroughly.
3. Incubate at 55°C for 3 hr. Vortex briefly every 20-30 min during incubation, or incubate with shaking.
4. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
5. Centrifuge at 10,000 x g for 3 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
6. Add 250 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.
7. Incubate with shaking at RT for 5 min.
8. 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.
9. 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.
10. 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.
11. Repeat step 10 for a second wash step.
12. 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.
13. Add 30-100 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.
14. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
15. Store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Sperm Sample

1. Vortex sperm sample, and transfer 150 µL sample into a 1.5 mL microcentrifuge tube.
2. Add 100 µL Buffer ATL, 20 µL Proteinase K solution, and 10 µL DTT (1 M). Vortex to mix thoroughly.
3. Incubate at 55°C for 30-60 min. Vortex briefly every 10 min during incubation, or incubate with shaking.
4. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
5. Add 250 µL 100% isopropanol and 50 µL MagBinding Beads. Mix thoroughly by vortexing.
6. Incubate with shaking at RT for 5 min.
7. 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.
8. 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.
9. 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.
10. Repeat step 9 for a second wash step.
11. 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.
12. Add 30-100 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.
13. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
14. Store the DNA at -20ºC.