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EasyMag DNA Purification Kit from bacteria

Catalog Number: D120-1, D120-2

Table 1. Kit Components and Storage

Kit Component	D120-1 (50 preps)	D120-2 (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℃	
Lysozyme	100 mg	400 mg	-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 bacteria provides a rapid and easy method for the isolation of high-quality genomic DNA from a wide variety of gram-positive and negative bacterial species. This kit can be processed manually or on an automated platform. The buffer system is optimized to allow direct cell lysis followed by selective binding of DNA to the magnetic beads. Three rapid wash steps remove trace salts and protein contaminants, and finally, DNA is eluted in water or low ionic strength buffer. 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 65°C.
- Prepare RNase A solution (10 mg/mL).
- Prepare Lysozyme working solution: dissolve lysozyme at 10 mg/mL in TE Buffer, pH 8.0.

Purification Protocol for Gram-Negative Bacteria

1. Harvest cells (maximum 1 x 10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.
2. Resuspend pellet in 180 µL Buffer ATL.
3. Add 20 µL Proteinase K solution. Mix thoroughly by vortexing, and incubate at 65°C for 30 min or until the cells are completely lysed. Vortex occasionally during incubation to disperse the sample.
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 15 min.
5. Add 200 µL Buffer DL to the sample, and mix thoroughly by vortexing. Then add 200 µL 100% isopropanol and 50 µL MagBinding Beads, and mix again thoroughly by vortexing.

   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 and isopropanol. This precipitate does not interfere with the DNA recovery.
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.

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Purification Protocol for Gram-Positive Bacteria

1. Harvest cells (maximum 1 x 10^9 cells) in a microcentrifuge tube by centrifuging for 10 min at 5000 x g (7500 rpm). Discard supernatant.
2. Resuspend bacterial pellet in 180 µL Lysozyme working solution.
3. Incubate for at least 30 min at 37°C.
4. Add 20 µL Proteinase K solution and 200 µL Buffer DL. Mix thoroughly by vortexing, and incubate at 65°C for 30 min or until the cells are completely lysed. Vortex occasionally during incubation to disperse the sample.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50°C to dissolve.
5. (*Optional*) If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL). Mix by vortexing, and incubate at RT for 15 min.
6. Add 200 µL 100% isopropanol and 50 µL MagBinding Beads to the sample, and mix thoroughly by vortexing.

   Note: A precipitate may form upon the addition of isopropanol. This precipitate does not interfere with the DNA recovery.
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 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.

Troubleshooting

Problem	Possible cause and suggestions
Low yield	• 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. • Loss of MagBinding Beads during operation: Avoid disturbing MagBinding Beads during aspiration. • DNA remains bound to MagBinding Beads: Increase elution volume and incubation time.
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.