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EasySC DNA Purification Kit from bacteria

Catalog Number: D119-1, D119-2

Table 1. Kit Components and Storage

Kit Component	D119-1 (50 preps)	D119-2 (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℃	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

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

Product Description

EasySC 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 uses an optimized lysis condition and up to 1 x 10^9 bacterial cells can be processed for each column. The buffer system is optimized to allow direct cell lysis followed by selective binding of DNA to the DNA column membrane. Simple centrifugation processing completely removes contaminations and enzyme inhibitors such as proteins and divalent cations, and allows simultaneous processing of multiple samples in parallel. 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 ethanol (96-100%), 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 ethanol. This precipitate does not interfere with the DNA recovery.
6. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 5 into the DNA Mini column including any precipitates that may have formed. Centrifuge at 10,000 x g for 1 min.
7. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   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. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
11. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
12. Discard the column and 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 ethanol (96-100%) to the sample, and mix thoroughly by vortexing.

   Note: A precipitate may form upon the addition of ethanol. This precipitate does not interfere with the DNA recovery.
7. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 6 into the DNA Mini column including any precipitates that may have formed. Centrifuge at 10,000 x g for 1 min.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   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. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
13. Discard the column and store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Column clogged	• Incomplete lysis: Extend incubation time to obtain complete lysis. • Too much sample: Reduce sample amount. • Incomplete removal of cell wall: Add more lysozyme or extend the incubation time.
Low yield	• Improper washing: Buffer AW1, buffer AW2 must be diluted with absolute ethanol before use. • Poor elution: Repeat elution with increased elution volume. Incubate columns at 65°C for 5 minutes with Buffer AE. • Column matrix lost binding capacity during storage: Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
Poor performance in downstream applications	• Salt contamination: Repeat Buffer AW2 wash twice. • Ethanol contamination: Incubate the column at 56°C for 5 min to dry the membrane completely.