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EasySC Gel DNA Extraction Kit

Catalog Number: D104-1, D104-2

Table 1. Kit Components and Storage

Kit Component	D104-1 (50 preps)	D104-2 (200 preps)	Storage	Stability
Buffer GDB	25 mL	100 mL	RT	The product is stable for one year when stored as directed.
Buffer PW*	15 mL	2×25 mL	RT	
Elution buffer	15 mL	25 mL	RT	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

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

Product Description

EasySC Gel DNA Extraction Kit uses the proprietary spin-column technology to purify DNA fragments ranging from 100 bp to 10 kb from all grades of agarose gels with high recovery (> 80%). The kit uses a specialized binding buffer system that dissolves the gel slice and binds to the spin column. The bind step is followed by three rapid wash steps and DNA is eluted with elution buffer. Purified DNA is ready for a variety of downstream applications such as ligations, PCR amplification, restriction enzyme digestion, cloning, and various labeling reactions.

Features

- ❖ Rapid – DNA recovery from an agarose gel in 15 min.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Things to do before starting

- Buffer GDB may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer PW is 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 50°C.

Purification Protocol

1. Perform agarose gel electrophoresis to fractionate DNA fragments. It is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.
2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.
3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.1 g will have a volume of 0.1 mL.

4. Add 1.5 volume Buffer GDB. For recovery of DNA fragment smaller than 200 bp, add 3 volumes Buffer GDB.

   Note: Buffer GDB may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.

5. Incubate at 50°C for 10 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.
6. Add one gel volume of isopropanol, and vortex for 10-20 seconds.
7. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer no more than 700 µL DNA/agarose solution from step 6 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
8. Discard the filtrate and reuse the collection tube. Repeat step 7 until the entire sample has been transferred to the column.
9. Add 200 µL of Buffer GDB to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
10. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer PW to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.

    Note: Buffer PW must be diluted with absolute ethanol according to the bottle label before use.

11. Repeat step 10 for a second wash step.
12. 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.

13. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube, add 30-50 µL Elution Buffer directly to the center of column matrix. Let sit at RT for 2 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.

14. Discard the column and store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Column clogged	• Agarose gel does not completely dissolve: Allow gel to completely melt. Add more Buffer GDB if necessary.
Low yield	• Agarose gel does not completely dissolve: Allow gel to completely melt. Add more Buffer GDB if necessary. • Too little Buffer GDB added to gel: Volume of agarose gel slice determined incorrectly. Add enough Buffer GDB as instructed. • Buffer PW is not diluted with ethanol: Buffer PW must be diluted with absolute ethanol before use.
Poor performance in downstream applications	• Salt contamination: add buffer Buffer PW to the column, let sit at RT for 2 min, then centrifuge. • Ethanol contamination: after centrifuging the empty DNA Mini Column at 12,000 x g for 3 min, open cap and let sit at RT for 5-10 min to completely dry the membrane.