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Product Information Sheet

Product Name: EB Pure Agarose Gel Extraction Kit *

Concentration: Not Applicable

Storage and Handling:

Store at room temperature upon arrival. Ensure that the bottle of Binder Buffer (XP2) is tightly capped when not in use. If any precipitates form in the buffers, warm at 37°C to dissolve.

Ordering Information:

Item Number	Preparation	Number of Tubes and Volumes
EB-AGEK-50	50	EB-AGEK Elution Buffer: 1x10mL, EB-AGEK SPW Wash Buffer: 1x25mL, EB-AGEK Binding Buffer (XP2): 1x40mL, EB-AGEK 2mL Collection Tubes: 50, EB-AGEK High-Bind DNA Mini Columns: 50
EB-AGEK-200	200	EB-AGEK Elution Buffer: 1x20mL, EB-AGEK SPW Wash Buffer: 3x25mL, EB-AGEK Binding Buffer (XP2): 1x150mL, EB-AGEK 2mL Collection Tubes: 200, EB-AGEK High-Bind DNA Mini Columns: 200

Product Description:

EB Pure Agarose Gel Extraction Kit is designed for extracting desired DNA fragments from Agarose Gel. The key to this system is the high binding matrix that specifically, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Then nucleic acids are easily eluted with deionized water or a low salt buffer. This kit is used to recover DNA fragments between 70 bp and 20 kb with yields exceeding 85%. The recovered DNA is suitable for ligations, PCR, sequencing, restriction digestion, or various labeling reactions.

Additional Materials and Equipment to be Supplied by User:

- Heat Block or water batch capable of 60°C
- Microcentrifuge capable of at least 13,000 x g
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 5M Sodium Acetate, pH 5.2
- Optional: Sterile deionized water
- Optional: Vacuum Manifold

Preparation Procedure:

Note: The yellow color of the Binding Buffer (XP2) signifies a pH of ≤ 7.5.

Set heating block or water bath to 60°C

Prepare SPW Wash Buffer:

- For EB-AGEK-50 Kits add 100mL of 100% Ethanol to the SPW Wash Buffer and indicate addition on the bottle.
- For EB-AGEK-200 Kits add 100mL of 100% Ethanol to each SPW Wash Buffer and indicate addition on the bottle.

Procedure Options:

Centrifugation Protocol – Listed below and in Lab Manual available online at empiricalbioscience.com

Vacuum Protocol – Listed in the Lab Manual available online at empiricalbioscience.com

Enzymatic Reaction Protocol – Listed in the Lab Manual available online at empiricalbioscience.com

Centrifugation Protocol

☐ 1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product. Excise the fragment of interest.

☐ 2. Determine the volume of your gel slice by weighing it in a clean 1.5mL microcentrifuge tube. Assuming a density of 1g/mL, a 0.3g gel slice will have a volume of 0.3mL.

☐ 3. Add 1 volume Binding Buffer (XP2). Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

☐ 4. Insert a High-Bind DNA Mini Column into a 2mL Collection Tube (provided).

☐ 5. Add no more than 700µL DNA/agarose solution from Step 3 to the High-Bind DNA Mini Column. Centrifuge at 10,000 x g for 1 minute at room temperature. Discard the filtrate and reuse the collection tube.

☐ 6. Repeat Step 5 until all of the sample has been transferred to the column.

☐ 7. Add 300µL Binding Buffer (XP2). Centrifuge at maximum speed (≥13,000 x g) for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

☐ 8. Add 700µL SPW Wash Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

☐ **Optional**: Repeat Step 8 for a second SPW Wash Buffer wash step.

☐ 9. Centrifuge the empty column at maximum speed for 2 minutes to dry the column. This step is critical for removal of trace ethanol that may interfere with downstream applications. Transfer the High-Bind DNA Mini Column into a clean 1.5mL microcentrifuge tube (not provided).

☐ 10. Add 30-50µL Elution Buffer or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute.

☐ 11. Store DNA at -20°C.