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Product Information Sheet

Product Name: EB Pure Tissue DNA Kit *

Concentration: Not Applicable

Storage and Handling:

Store at room temperature upon arrival. OB Protease Solution can be stored at room temperature for up to 6 months upon receipt. For long-term storage (>6 months), store at 2-8°C. Check buffers for precipitates before use. Redissolve any precipitate by warming to 37°C.

Ordering Information:

Item	Preparations	Number of Tubes and Volumes
EB-TDK-50	50	EB-TDK OB Protease Solution: 1x1.5mL, EB-TDK Elution Buffer: 1x30mL, EB-TDK DNA Wash Buffer: 1x15mL, EB-TDK HBC Buffer: 1x25mL, EB-TDK TL Buffer: 1x20mL, EB-TDK BL Buffer: 1x20mL, EB-TDK 2mL Collection Tubes: 100, EB-TDK High Bind DNA Mini Columns: 50
EB-TDK-200	200	EB-TDK OB Protease Solution: 1x6mL, EB-TDK Elution Buffer: 2x50mL, EB-TDK DNA Wash Buffer: 3x25mL, EB-TDK HBC Buffer: 1x80mL, EB-TDK TL Buffer: 1x60mL, EB-TDK BL Buffer: 1x60mL, EB-TDK 2mL Collection Tubes: 400, EB-TDK High Bind DNA Mini Columns: 200

Product Description:

EB Pure Tissue DNA Kit is a convenient system for the fast and reliable purification of genomic DNA from animal tissue. The key to this system is the High-Bind matrix that specifically, but reversibly, binds DNA under optimized conditions allowing proteins and other contaminants to be removed. The DNA is easily eluted with deionized water or a low salt buffer. There is no need for phenol/chloroform extractions, and time-consuming steps such as alcohol precipitation are eliminated. This kit is used to purify genomic DNA which is then ready for applications such as PCR, Southern blotting, and restriction enzyme digestion.

Additional Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Shaking water baths, heat blocks, or incubators capable of 55-70°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- PBS (for cultured cells)
- Optional: RNase stock solution (100 mg/mL)

Preparation Procedure:

Prepare DNA Wash Buffer and HBC Buffer:

- For EB-TDK-50 Kits add 60mL of 100% Ethanol to the DNA Wash Buffer Bottle and indicate addition on the bottle.
- For EB-TDK-50 Kits add 10mL of 100% Isopropanol to the HBC Buffer Bottle and indicate addition on the bottle.
- For EB-TDK-200 Kits add 100mL of 100% Ethanol to each DNA Wash Buffer Bottle and indicate addition on the bottle.
- For EB-TDK-200 Kits add 32mL of 100% Isopropanol to the HBC Buffer Bottle and indicate addition on the bottle.
- Set water baths, heat blocks, or incubators to 55°C and 70°C, Chill PBS to 4°C (for cultured cells), Heat Elution Buffer to 70°C.

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

Centrifugation Protocol

☐ 1. **For Tissue:**
- Mince up to 30 mg tissue and transfer to a 1.5 mL centrifuge tube.
- Add 200 µL of TL Buffer and 25 µL OB Protease Solution. Vortex to mix thoroughly.
- Incubate at 55°C for 3 hours up to overnight. If shaking water bath is not available, vortex every 20-30 minutes.
- Optional: Remove RNA by adding 4 µL RNase A and incubating 2 minutes at room temperature.
- Centrifuge at maximum speed for 5 minutes. Transfer the supernatant to a sterile 1.5mL microcentrifuge tube. Do not disturb the pellet.

For Cultured Cells:
- Prepare the cell suspension in one of the following ways:
  - Frozen Cells: Thaw and pellet by centrifugation. Wash with cold PBS.
  - Cells grown in suspension: Pellet 5 x 10^6 in a centrifuge tube. Wash with cold PBS.
  - Cells grown in a monolayer: Harvest cells by scraping or with trypsin. Wash twice with cold PBS.
- Resuspend in 200 µL cold PBS. Add 25 µL OB Protease Solution. Vortex to mix thoroughly.
- Optional: Remove RNA by adding 4 µL RNase A and incubating 2 minutes at room temperature.

☐ 2. Add 220 µL BL Buffer. Vortex to mix thoroughly. Note: A wispy precipitate may appear and does not affect DNA recovery.

☐ 3. Incubate at 70°C for 10 minutes. Add 220 100% ethanol.

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

☐ 5. Transfer the entire sample to the Mini Column, including any precipitate. Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the Collection Tube.

☐ 6. Add 500 µL HBC Buffer diluted with 100% isopropanol. Centrifuge at maximum speed for 30 seconds.

☐ 7. Insert the High Bind Mini Column into a new 2 mL Collection Tube (provided).

☐ 8. Add 700 µL DNA Wash Buffer diluted with 100% ethanol (See Bottle) to the column. Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the Collection Tube.

☐ 9. Repeat Step 8 for a second wash step.

☐ 10. Centrifuge at maximum speed for 2 minutes to dry the column.

☐ 11. Transfer the High-Bind Mini Column to a clean 1.5mL microcentrifuge tube (not provided).

☐ 12. Add 100-200 µL Elution Buffer. Incubate for 2 minutes at room temperature. Centrifuge at maximum speed for 1 minute.

☐ 13. Store eluted DNA at -70°C.