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Product Information Sheet

Product Name: EB Pure Plasmid Mini-Prep Kit *

Concentration: Not Applicable

Storage and Handling:

Store at room temperature upon arrival. Solution I once the RNase A has been added should be stored 2-8ºC. Solution II must be tightly capped when not in use.

Ordering Information:

Item	Preparations	Number of Tubes and Volumes
EB-PMK-50	50	EB-PMK Solution 1: 1x20mL, EB-PMK Solution 2: 1x20mL, EB-PMK Solution 3: 1x20mL, EB-PMK HBC Buffer: 1x25mL, EB-PMK DNA Wash Buffer: 1x15mL, EB-PMK Rnase A: 1x100uL, EB-PMK Elution Buffer: 1x10mL, EB-PMK High-Bind DNA Mini Columns: 50, EB-PMK 2mL Collection Tubes: 50
EB-PMK-200	200	EB-PMK Solution 1: 1x60mL, EB-PMK Solution 2: 1x60mL, EB-PMK Solution 3: 1x60mL, EB-PMK HBC Buffer: 1x80mL, EB-PMK DNA Wash Buffer: 3x25mL, EB-PMK Rnase A: 1x400uL, EB-PMK Elution Buffer: 1x30mL, EB-PMK High-Bind DNA Mini Columns: 200, EB-PMK 2mL Collection Tubes: 200

Product Description:

EB Pure Plasmid Mini-prep Kit combine the power of High-Bind technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality DNA in less than 30 minutes. The key to this system is the High-Bind 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. The EB Pure Plasmid Mini-prep Kit is used to isolate plasmid DNA from 1-5mL cultures. Purified plasmid DNA can be directly used for most downstream applications including automated fluorescent DNA sequencing and restriction enzyme digestion.

Additional Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x g
- Vortexer
- Nuclease-free 1.5 mL or 2mL microcentrifuge tubes
- Culture tubes
- 100% ethanol
- 100% isopropanol
- Optional: Sterile deionized water
- Optional: water bath or incubator capable of 70ºC
- Optional: 3M NaOH solution

Preparation Procedure:

1. Add the vial of Rnase A to the bottle of Solution I and store at 2-8ºC (50 and 200 prep size only)
2. Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37ºC.
3. Heat Elution Buffer to 70ºC if plasmid DNA is >10 kb.
4. Set water baths, heat blocks, or incubators to 55ºC and 70ºC.
5. Dilute DNA Wash Buffer with 100% Ethanol as follows and store at room temperature:
    a. EB-PMK-50 add 60mL of 100% Ethanol
    b. EB-PMK-200 add 100mL of 100% Ethanol
6. Prepare HBC Buffer with 100% isopropanol as follows and store at room temperature:
    a. EB-PMK-50 add 10mL of 100% Isopropanol
    b. EB-PMK-200 add 32mL of 100% Isopropanol

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

***This product is intended for Research Use Only. This product is manufactured under ISO13485:2016 Quality System Requirements and is available for use as a Raw Material for use in IVD applications. Please contact Empirical Bioscience for further details.**

For MSDS and Certificate of Analysis please visit www.empiricalbioscience.com

Centrifugation Protocol

☐ 1. Grow 1-5mL culture overnight in a 10-20mL culture tube.

☐ 2. Centrifuge at 10,000 x g for 1 minute at room temperature. Decant or aspirate and discard the culture media.

☐ 3. Add 250µL Solution I mixed with RNase A (see above for instructions). Vortex to mix thoroughly. Transfer suspension into a new 1.5mL microcentrifuge tube.

☐ 4. Add 250µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary. Avoid vigorous mixing and do not exceed a 5 minute incubation.

☐ 5. Add 350µL Solution III. Immediately invert several times until a flocculent white precipitate forms. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

☐ 6. Insert a High-Bind DNA Mini Column into a 2mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100µL 3M NaOH to the High-Bind DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

☐ 7. Transfer the cleared supernatant from Step 6 by CAREFULLY aspirating it into the High-Bind DNA Mini Column. Centrifuge at maximum speed for 60 seconds. Discard the filtrate and reuse the collection tube.

☐ 8. Add 500µL HBC Buffer diluted with isopropanol (see above for instruction).

☐ 9. Centrifuge at maximum speed for 60 seconds. Discard the filtrate and reuse the collection tube.

☐ 10. Add 700µL DNA Wash Buffer diluted with ethanol (see above for instructions). Centrifuge at maximum speed for 30 seconds. Discard the filtrate and reuse the collection tube.

☐ 11. Repeat Step 10 for a second DNA Wash Buffer wash step.

☐ 12. Centrifuge the empty High-Bind DNA Mini 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.

☐ 13. Transfer the High-Bind DNA Mini Column into a nuclease-free 1.5mL microcentrifuge tube.

☐ 14. Add 30-100µL Elution Buffer or sterile deionized water. Let sit at room temperature for 60 seconds. Centrifuge at maximum speed for 60 seconds.

☐ 15. Store eluted DNA at -20°C.

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