

Product Name: EB Pure Plasmid Mini-prep Kit*

Item No.:

Item Number	Preparations
SS-EB-PMK-5	5
EB-PMK-50	50
EB-PMK-200	200

Storage and Handling:

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

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.

Kit contents:

Component	SS-EB-PMK-5	EB-PMK-50	EB-PMK-200
High-Bind DNA Mini Columns	5	50	200
2mL Collection Tubes	5	50	200
Solution I	3mL	20mL	60mL
Solution II	3mL	20mL	60mL
Solution III	3mL	20mL	80mL
HBC Buffer	4mL	25mL	80mL
DNA Wash Buffer	1.5mL	15mL	3 x 25mL
RNase A	Pre-Added	100µL	400µL
Elution Buffer	1.5mL	10mL	30mL

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 70°C
- Optional: 3M NaOH solution

Preparation Procedure:

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8°C (50 and 200 prep size only)
2. Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37°C.
3. Heat Elution Buffer to 70°C if plasmid DNA is >10 kb.
4. Set water baths, heat blocks, or incubators to 55°C and 70°C.
5. Dilute DNA Wash Buffer with 100% Ethanol as follows and store at room temperature:

Kit	SS-EB-PMK-5	EB-PMK-50	EB-PMK-200
100% Ethanol to be Added	6mL	60mL	100mL

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6. Prepare HBC Buffer with 100% isopropanol as follows and store at room temperature:

Kit	SS-EB-PMK-5	EB-PMK-50	EB-PMK-200
100% Isopropanol to be Added	1.6mL	10mL	32mL

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. 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 ($\geq 13,000 \times g$) 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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