Empirical bioscience

Product Information Sheet and Protocol

Product Name: EB Pure PCR Purification Kit*

Item No.:

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

Storage and Handling:
Store at room temperature upon arrival. Dissolve any precipitates that may form in the CP buffer by warming the solution at 37°C and gently shaking.

Product Description:
EB Pure PCR Purification Kit is a convenient system for the fast and reliable purification of PCR products. 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. This kit is used to recover DNA bands from 100 bp to 10 kb free of oligonucleotides, nucleotides, and polymerase with yields exceeding 80%. Purified DNA can be directly used for most downstream applications including T-A ligations, PCR sequencing, restriction enzyme digestion, or various labeling reactions.

Kit contents:

Component	SS-EB-PPK-5	EB-PPK-50	EB-PPK-200
High-Bind DNA Mini Columns	5	50	200
2mL Collection Tubes	5	50	200
CP Buffer	5mL	40mL	150mL
DNA Wash Buffer	1.5mL	15mL	3 x 25mL
Elution Buffer	5mL	10mL	20mL

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
100% ethanol
For fragments < 200 bp, 100% isopropanol
Vortexer
Optional: Sterile deionized water or TE Buffer
Optional: Compatible vacuum manifold

Preparation Procedure:
Prepare DNA Wash Buffer according the table below:

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

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Centrifugation Protocol

- ☐ 1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.
- ☐ 2. Determine the volume of your PCR reaction.
- ☐ 3. Transfer the sample into a clean 1.5mL microcentrifuge tube (not provided).
- ☐ 4. Add 4-5 volumes CP Buffer. For PCR products smaller than 200 bp, add 5 volumes CP Buffer and 0.4 volumes 100% isopropanol. **Note**: Volume refers to the size of your PCR reaction. For example, if your PCR reaction is 100µL and is smaller than 200 bp, you would use 500µL CP Buffer and 40µL isopropanol.
- ☐ 5. Vortex to mix thoroughly. Briefly centrifuge to collect any drops from the inside of the lid.
- ☐ 6. Insert a High-Bind DNA Mini Column into a 2mL Collection Tube (provided).
- ☐ 7. Add the sample from Step 5 to the High-Bind DNA Mini Column.
- ☐ 8. Centrifuge at maximum speed (≥13,000 x g) for 1 minute at room temperature.
- ☐ 9. Discard the filtrate and reuse collection tube.
- ☐ 10. Add 700µL DNA Wash Buffer. **Note**: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see above preparation section for instructions.
- ☐ 11. Centrifuge at maximum speed for 1 minute.
- ☐ 12. Discard the filtrate and reuse collection tube.
- ☐ 13. Repeat Steps 10-12 for a second DNA Wash Buffer wash step.
- ☐ 14. Centrifuge the empty High-Bind DNA Mini Column at maximum speed for 2 minutes to dry the column. **Note**: This step is critical for removal of trace ethanol that may interfere with downstream applications.
- ☐ 15. Transfer the High-Bind DNA Mini Column into a clean 1.5mL microcentrifuge tube (not provided).
- ☐ 16. Add 30-50µL Elution Buffer, TE Buffer, or sterile deionized water directly to the center of column matrix.
- ☐ 17. Let sit at room temperature for 2 minutes.
- ☐ 18. Centrifuge at maximum speed for 1 minute. **Note**: This represents approximately 80-90% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration
- ☐ 19. Store DNA at -20°C.

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