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One page protocol

AccuPrep® PCR/Gel Purification Kit (K-3037G)

Ⅰ Before You Begin

1) Add indicated volume of isopropanol (not provided) to PB Buffer before use (see bottle label).
2) We recommend to use ≤ 1% agarose gel for gel purification.
3) Pre-heat EA Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.
4) The protective seal in BSTB Solution should be completely removed. BSTB Solution may be discolored, but it does not affect nucleic acid extraction.
5) Add adequate amount of **absolute ethanol (not provided)** to **WB2 Buffer** and **BSTB Solution**, respectively (see bottle label).

Ⅱ Fragment DNA purification from PCR product

1) Add **5 volumes** of **PB Buffer** to PCR product (if the PCR product is 20 µl, add 100 µl of PB Buffer).
2) Add absolute isopropanol (not provided) by the same volume as PCR product and mix immediately by using pipette or inverting.
3) Add **100 µl** of **BSTB Solution** to the Binding column tube (fitted in a collection tube) and centrifuge for **30 sec** at **13,000 rpm**.
4) Discard the solution from the collection tube and reuse the collection tube.
5) Transfer the mixture from step 2 to a Binding column in a collection tube.
6) Close the lid and centrifuge at **14,000 rpm** for **1 min**.
7) Discard the flow-through and re-assemble the Binding column with the collection tube.
8) Add **500 µl** of **WB2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **1 min**. Discard the flow-through and re-assemble the Binding column with the collection tube.
9) **Repeat step 8**.
10) Centrifuge once more at **14,000 rpm** for **1 min** to completely remove residual ethanol, and make sure that there is no droplet clinging to the bottom of the Binding column.
11) Transfer the Binding column tube to a new 1.5 ml tube for elution, add **30 µl** of **EA Buffer** onto the Binding column tube, and wait for at least 1 min at RT (15-25°C).
12) Centrifuge at **14,000 rpm** for **1 min** to elute.

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III Fragment DNA purification from Agarose gel

1) Visualize the band in agarose gel stained with any nucleic acid staining chemicals and cut the gel around the target DNA band using a scalpel blade.
2) The maximum amount of gel slice per each sample is **< 400 mg and add 3 times the volume** of **FB Buffer** according to the weight of the gel slice (If the weight of gel slice is 200 mg, add 600 µl of FB Buffer).
3) Incubate at **50°C** for **10 min** and mix by inverting the tube every 2-3 min during the incubation. After dissolution of the gel slice, check if the color of the mixture is yellow which indicates pH ≤ 7.5. If the color of the mixture is orange or red, add 10 µl of 3M sodium acetate (not provided, pH 5.0) and mix.
4) Add **absolute isopropanol (not provided)** by the same volume as dissolved gel mixture and mix immediately by using pipette or inverting.
5) Add **100 µl** of **BSTB Solution** to the Binding column tube (fitted in a collection tube) and centrifuge for **30 sec** at **13,000 rpm**.
6) Discard the solution from the collection tube and reuse the collection tube.
7) Transfer the mixture from step 4 to a Binding column in a collection tube.
8) Close the lid and centrifuge at **14,000 rpm** for **1 min**.
9) Discard the flow-through and re-assemble the Binding column with the collection tube.
10) Add **500 µl** of **WB2 Buffer** without wetting the rim, close the tube, and centrifuge at **14,000 rpm** for **1 min**. Discard the flow-through and re-assemble the Binding column with the collection tube.
11) **Repeat step10**.
12) Centrifuge once more at **14,000 rpm** for **1 min** to completely remove residual ethanol, and make sure that there is no droplet clinging to the bottom of the Binding column.
13) Transfer the Binding column tube to a new 1.5 ml tube for elution, add **30 µl** of **EA Buffer** onto the Binding column tube, and wait for at least 1 min at RT (15-25°C).
14) Centrifuge at **14,000 rpm** for **1 min** to elute.

※ For more information, please visit www.bioneer.com and refer to the User Guide of this kit.