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One page protocol

AccuPrep® PCR/Gel Purification Kit (K-3037, K-3038)

Ⅰ Before You Begin

1) Please prepare absolute isopropanol (not provided) before purification.
2) We recommend to use ≤1% concentration of agarose gel for gel purification.
3) Did you add adequate amount of isopropanol to PB Buffer?
4) The protective seal in BST Solution should be completely removed. BST Solution may be discolored, but it does not affect nucleic acid extraction.

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 **1 PCR product volume** of **absolute isopropanol** and mix immediately by using pipette or inverting.
3) Add **100 µl** of **BST Solution** to the Binding column tube (fit 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 to a Binding column in a 2 ml 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 **W2 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 check 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℃).
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 DNA band of interest using a scalpel blade.
2) The maximum amount of gel slice per each sample is **~400 mg**, Add **3 volumes** of **FB Buffer** to 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 the color of the mixture is yellow that indicates pH ≤ 7.5. If the color of the mixture is orange or red, add 10 µl of 3M sodium acetate (pH 5.0) and mix.
4) Add **1 gel volume** of **absolute isopropanol** and mix immediately by using pipette or inverting.
5) Add **100 µl** of **BST Solution** to the Binding column tube (fit 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 to a Binding column in a 2 ml 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 **W2 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 check 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's Guide of this kit.