

One page protocol

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

(Recommendation

- 1) Please prepare absolute isopropanol (not provided) before purification.
- 2) We recommend to use ≤1% concentration of agarose gel for gel purification.
- 3) For high-purity DNA extraction from >200 mg of agarose gel (max. 400 mg), please use WB1 Buffer in washing step.

II) Fragment DNA purification from PCR product

- 1) Add 3 volumes of FB Buffer to PCR product (If the PCR product is 20 μ l, add 60 μ l of FB Buffer)
- 2) Add 1 volume of absolute isopropanol and mix immediately by using pipette or inverting.
- 3) Transfer the mixture to a binding column in a 2 ml collection tube.
- 4) Close the lid and centrifuge at 14,000 rpm for 1 min.
- 5) Discard the flow-through and re-assemble the binding column with the collection tube.
- 6) 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.
- 7) Repeat step 6.
- 8) 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.
- 9) 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).
- 10) Centrifuge at 14,000 rpm for 1 min to elute.



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(III) Fragment DNA purification from Agarose gel

- 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 \sim 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 μ l of FB Buffer)
- 3) Incubate at 50°C for 10 min and mix by inverting the tube every 2-3 min during the incubation.
- 4) Add 1 volume of absolute isopropanol and mix immediately by using pipette or inverting.
- 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.
- Discard the flow-through and re-assemble the binding column with the collection tube.
- 8) (Optional) Add 500 µl of WB1 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) 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.
- 10) Repeat step 9.
- 11) 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.
- 12) 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).
- 13) Centrifuge at 14,000 rpm for 1 min to elute.