E.Z.N.A.® Gel Extraction Kit

Revision No: 2.1

Quick Guide

Please visit www.omegabiotek.com for a downloadable user manual containing additional protocols, troubleshooting tips, and ordering information.

Product	D2500-00	D2500-01	D2500-02
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
XP2 Binding Buffer	5 mL	40 mL	150 mL
SPW Buffer	5 mL	25 mL	3 x 25 mL
Elution Buffer	2 mL	30 mL	30 mL

Supplied by user:

- Tabletop microcentrifuge capable of 13,000 x *g*
- Heat block or water bath capable of 60°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 5M sodium acetate, pH 5.2
- Optional: sterile deionized water
- Optional: vacuum manifold

Before starting:

- Prepare SPW Buffer according to the directions on the bottles.
- Heat the heat block or water bath to 60°C.

DNA Purification from Agarose Gels - Centrifugation Protocol

BIND

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product. Excise the fragment of interest.
2. Determine the volume of your gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1g/mL, a 0.3 g gel slice will have a volume of 0.3 mL.
3. Add 1 volume XP2 Binding Buffer. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

   Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.
4. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube (provided).
5. Add no more than 700 µL DNA/agarose solution from Step 3 to the HiBind® DNA Mini Column. Centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the filtrate and reuse the collection tube.
6. Repeat Step 5 until all of the sample has been transferred to the column.
7. Add 300 µL XP2 Binding Buffer. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature. Discard the filtrate and reuse collection tube.

WASH

8. Add 700 µL SPW Buffer diluted with 100% ethanol (see the bottle for instructions). Centrifuge at maximum speed for 1 minute. Discard the filtrate and reuse the collection tube.

Optional: Repeat Step 8 for a second SPW Buffer wash step.

9. Centrifuge the empty 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. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).

ELUTE

10. Add 30-50 µL Elution Buffer or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed for 1 minute.
11. Store DNA at -20°C.

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DNA Purification from Agarose Gels - Vacuum Protocol

BIND

1. Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.
2. Excise the fragment of interest.
3. Determine the volume of your gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, a 0.3 g gel slice will have a volume of 0.3 mL.
4. Add 1 volume XP2 Binding Buffer. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

   Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.
5. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.
6. Add no more than 700 µL DNA/agarose solution from Step 4 to the HiBind® DNA Mini Column. Switch on the vacuum source to draw the sample through the column. Turn off the vacuum.
7. Repeat Step 6 until all of the sample has been transferred to the column.

WASH

8. Add 300 µL XP2 Binding Buffer. Switch on the vacuum source to draw the XP2 Binding Buffer through the column. Turn off the vacuum.
9. Add 700 µL SPW Buffer diluted with 100% ethanol (see the bottle for instructions). Switch on the vacuum source to draw the SPW Buffer through the column. Turn off the vacuum.

Optional: Repeat Step 9 for a second SPW Buffer wash step.

10. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).
11. Centrifuge the empty HiBind® 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.

ELUTE

12. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube (not provided).
13. Add 30-50 µL Elution Buffer or sterile deionized water directly to the center of column matrix. Let sit at room temperature for 2 minutes. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute.
14. Store DNA at -20°C.