GSure® Gel Extraction Kit

#G4629C 250 preparations

Store at Room Temperature

Procedure:

1. Excise the agarose gel slice containing the desired DNA fragment. Remove excess agarose to minimize the gel slice.
2. Transfer the gel slice into a microfuge tube (not provided)
3. Weigh the gel slice (maximum 300mg/isolation) in a fine balance. For exact weight of the slice, tare the balance with empty microfuge tube.
4. Add 3 volume of GGMB (Gel melting buffer) buffer to the gel slice (consider 1mg of gel slice as 1µl).
5. Incubate at **75°C** for 15 minutes. **GGMB (Gel melting buffer) of GSure Gel extraction kit is a highly improved and efficient buffer that does not contain any traces of Guanidinium Thiocyanate thus assuring most purified DNA for downstream processing.**
6. Wait until the gel slice has been completely dissolved. During incubation, vortex the tube every 2-3 minutes. GGMB contains a color indicator which will turn pink if pH of the solution increases. High pH inhibits DNA binding with column.

   If the color changes to pink, add 3M sodium acetate pH 5.2 (not provided), until the color changes to straw yellow again.
7. After the gel slice dissolved completely add 1 gel volume of isopropanol.
8. Apply total solution into the GMini Spin column.
9. Centrifuge at 13,000 rpm (~8500xg) for 30–60 s. Discard the flow-through.
10.
11. Wash GMini Spin Column by adding 600µl Membrane Wash Buffer and centrifuging for 30–60 s as previously.
12.
13. Discard the flow-through.
14. Repeat washing step.
15. Discard the flow-through, and centrifuge for an additional 2 min to remove residual wash buffer from membrane.

    This step is extremely important to ensure complete removal of ethanol. Presence of ethanol in purified DNA may inhibit successive enzymatic reactions.
16. Place the GMini Spin Column in a clean 1.5 ml microcentrifuge tube (not provided). To elute DNA, add 50 µl Nuclease-free Water (provided) to the center of each GMini Spin Column, let stand for 2 minutes, and centrifuge for 1 min at maximum speed (~8500Xg) on a table top microcentrifuge.
17. Discard the column and collect the eluted DNA present in microcentrifuge tube.

Kit Contents:

GGMB Buffer	**: 150ml X 2**
Membrane Wash Buffer	**: 75ml X 2**
Spin Column	**: 50 pcs X 5**
Nuclease-free Water	**: 15ml**

Reconstitution of Membrane Wash Buffer:

Before using the kit for first time, add 112.5 ml of absolute ethanol (molecular biology grade) with the provided wash buffer each. Mix thoroughly by shaking. Once ethanol added, tighten the cap properly after each use.