BIOLOGICAL INDUSTRIES
ISRAEL BEIT HAEMEK LTD.
Kibbutz Beit Haemek 25115 Israel Tel. 972-(0)-4-9960595, Fax. 972-(0)-4-9968896, e-mail:info@bioind.com

Biological Industries DNA Isolation Kit

for 150-300 Isolations

The DNA Isolation Kit provides a convenient method for extracting DNA from agarose gels, isolating plasmid DNA from mini-preps, as well as for concentration of DNA without ethanol precipitation. (Vogelstein, B. And Gillespie, D., 1979, Proc. Natl. Acad. Sci. USA, 76,615-619).

Cat. No.: **20-200-300**
Store at: **2-8°C**

Kit Reagents

Glass Powder Suspension, 1.5ml
Sodium Iodide Solution (6M), 120ml
Concentrated Wash Solution, 25ml

Preparation and Storage of Reagents

1. Glass Powder Suspension: Store tightly sealed with parafilm at 2-8°C. Mix well before using. Dried-out glass powder suspension can be resuspended into molecular biology grade water, such that the glass powder suspension accounts for approximately two-thirds of the total volume.

2. Sodium Iodide Solution: Store at 2-8°C protected from light.

3. Concentrated Wash Solution: Before the first use add 225ml of sterile distilled water to 25ml concentrate, and then add 250ml of absolute ethanol. Store at -20°C in a glass bottle.

Protocol for DNA Isolation Kit

1 Extraction of DNA Fragments from Agarose

1.1 Excise DNA band from ethidium bromide stained gel with a razor blade using 312nm UV light.

1.2 Add 3 volumes of 6M NaI solution (provided with the kit) per gel slice volume to the vial containing the agarose piece. In the case of TBE-based gels, add ½ volume of 3M Sodium Acetate solution (pH=5.2) and 4.5 volumes of NaI solution to a given volume of gel slice. The final concentration of NaI should be at least 4M. Incubate at 55°C for 2-5 minutes, mix contents of the tube and return it to the water bath for an additional period of 1-2 minutes. At this point the agarose gel should be completely dissolved.

1.3 To the agarose:DNA:NaI solution add the glass powder suspension. Use 6µl well-mixed suspension for the first 2µg DNA and an additional µl for each 0.5µg DNA thereafter. Mix well and incubate at room temperature for 5 minutes. Mix every 1-2 minutes. With fragments smaller than 1000bp, incubate at 55°C to improve binding efficiency.

1.4 Microcentrifuge at maximum speed for 10 seconds and discard the supernatant solution.

1.5 Rinse the glass pellet with wash buffer. Use 50 volumes of wash buffer compared to the original glass powder volume. Resuspend the glass pellet by pipetting back and forth , or by gently flicking the tube. Use very gentle pipetting when working with large (>15kb) DNA pieces.

1.6 Repeat washing step two more times.

1.7 After the supernatant of the last wash has been removed, spin the tube again and remove as much of the wash buffer as possible using a micropipette tip.

1.8 Suspend the glass pellet in 1-2 volumes of the original glass suspension used for isolation. Use sterile distilled water or TE buffer (10mM Tris pH=7.5-8.0; 1mM EDTA). Elute for 3-5 minutes at 55°C with occasional mixing.

1.9 Microcentrifuge at maximum speed for 30-45 seconds. Carefully transfer the supernatant containing the eluted DNA to a new tube. An additional amount of 10-15% of recovered DNA can be obtained from the pellet by repeated elution.

2 Isolation of Plasmid DNA from Overnight Culture

2.1 Grow 2-3ml Transformant culture overnight.

2.2 Pour about 1.5ml into a microcentrifuge tube and pellet cells for 15-30 seconds at 12000 rpm.

2.3 Discard supernatant and resuspend in 100µl of ice cold TGE buffer (25mM Tris pH=8.0; 50mM glucose; 10mM EDTA).

2.4 Add 200µl of 0.2N NaOH+1% SDS solution. Mix, but do not vortex. Leave on ice.

2.5 Add 150µl of cold potassium acetate solution (3M with respect to potassium, 5M with respect to acetate).

2.6 Mix well, but do not vortex. Leave on ice for 3-5 minutes.

2.7 Microcentrifuge at maximum speed for 5 minutes.

2.8 Carefully transfer supernatant to a clean microcentrifuge tube.

2.9 Add 3 volumes of 6M NaI. Mix well.

2.10 Add 10µl of glass powder suspension. Mix well and incubate at room temperature for 5 minutes.

2.11 Microcentrifuge at maximum speed for 10 seconds and discard the supernatant solution.

2.12 Rinse the glass pellet with wash buffer. Use 50 excess volumes of wash buffer compared to the original glass powder volume. Resuspend the glass pellet by pipetting back and forth, or by gently flicking the tube.

2.13 Repeat washing step two more times.

2.14 After the supernatant of the last wash has been removed, spin the tube again and remove as much of the wash buffer as possible using a micropipette tip.

2.15 Suspend the glass pellet in 1-2 volumes of the original glass suspension used for isolation. Use sterile distilled water or TE buffer (10mM Tris pH=7.5-8.0; 1mM EDTA). Elute for 3-5 minutes at 55°C with occasional mixing.

2.16 Microcentrifuge at maximum speed for 30-45 seconds. Carefully transfer the supernatant containing the eluted DNA to a new tube. An additional amount of 10-15% of recovered DNA can be obtained from the pellet by repeated elution.