NORGEN BIOTEK CORPORATION

DNA Gel Extraction Kit

Short Protocol Card

1. Excising DNA From Gel

a. Run DNA fragment of interest on agarose gel[1].
b. Excise fragment from gel using a scalpel or razor blade. Remove as much excess agarose as possible, and minimize exposure of DNA to UV light.
c. Place the excised agarose into a sterile and pre-weighed 1.5 mL microcentrifuge tube.

2. Sample Preparation

a. Determine the weight of the gel slice.
b. Add 3 volumes of **Binding Solution** to 1 volume of gel (assuming 100 mg of gel equals 100 µL of **Binding Solution**)[2].
c. Incubate at 55°C for 10 minutes, or until completely dissolved. Vortex every 2 to 3 minutes to assist in dissolving[3].
d. Once gel slice is completely dissolved, add 1 gel volume of isopropanol and mix[4].

3. Binding DNA to Column

a. Apply the sample to a column[5] and centrifuge for one minute[6].
b. Discard the flowthrough and reassemble spin column with its collection tube.

4. Washing Bound DNA

a. Apply 500 µL of **Wash Solution**[7] to the column and centrifuge for one minute.
b. Discard the flowthrough and reassemble column and collection tube.
c. Repeat steps **4a** and **4b**.
d. Spin column for one additional minute in the collection tube, in order to dry completely.

5. Elution of Clean DNA

a. Place the column into one of the provided **Elution tubes**.
b. Add 30 µL of **Elution Buffer** to the center of the resin bed and centrifuge for one minute[8].

[1] It is recommended that fresh running buffer be used for the gel, as re-used buffer exhibits increased pH and may reduce DNA recovery from spin columns.
[2] For gels made with greater than 2% agarose, add 6 volumes of **Binding Solution**. For larger gel slices, cut the gel into smaller pieces to facilitate melting.
[3] It is important to dissolve agarose completely. For greater than 2% gels, an increased incubation time may be required.
[4] Do not centrifuge during this step of the protocol.
[5] The maximum volume that the reservoir can accommodate during each spin is 1 mL. If the sample volume exceeds this, repeat spin as necessary until the entire sample has been processed.
[6] All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g.
[7] **Wash Solution** is prepared by adding 45 mL of 95% ethanol to the supplied volume of **Wash Concentrate**, resulting in a final volume of 60 mL. The label on the bottle has a box that can be checked to indicate that the ethanol has been added.
[8] An additional elution may be performed if desired. Another 30 µL of **Elution Buffer** could be added to the column and centrifuged for one minute into a new **Elution Tube**, such that the initial elution is not diluted.

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