PCR-Products and GEL/DNA Purification Kit

Description

Materials Provided:

Component	100 Preps	5 Preps
*DNA Cleanup Binding Buffer	40 ml (addÂ **20 mlÂ** of 96-100% Isopropanol)	2 ml (add Isopropa
**DNA Wash Buffer	10 ml (addÂ **40 mlÂ** of 96-100% ethanol)	0.5 ml (a 100% eth
DNA Elution Buffer	10ml	1 ml
Mini Columns	100	5
Tubes as column inserts	100	5

Additional requirements:Â microcentrifige Tubes , (96-100%) absolute ethanol , (96-100%) isopropanol

Note:

*Add 96-100% isopropanol to DNA Cleanup Binding buffer as written on the bottle before using.

**Add 96-100% of ethanol to DNA Wash buffer as written on the bottle before using.

*You can use either Isopropanol or Ethanol in binding buffer but preference should be given toÂ Isopropanol as it gives better results.

Procedure:

1. Add 95% ethanol to DNA Wash Buffer prior to use in the ratio of 4: 1 (i.e. 4 volumes ofÂ Â Â Â Â Â Â Â Â Â Â Â 95% ethanol and one volume of DNA Wash
2. Dilute sample with DNA Cleanup Binding Buffer in specific ratio of sample: binding buffer depending the size of the

For example:

ssDNA (cDNA), sample to binding buffer ratio 1:6 dsDNA (< 2.5 kb), sample to binding buffer ratio 1:5 dsDNA (> 1.5 kb), sample to binding buffer ratio 1:2

For purifying DNA from gel slice:

Take 25 to 50 mg of gel slice into a clean microfuge tube. Add 500 ul binding buffer (containing Isopropanol) and warm the tube at 55Â°C for 15 minutes or more until the gel slice dissolves. Proceed to the next step â€“ loading directly into the spin column.

3. Mix well by pipetting up and down or by tapping the Do not vortex.
4. Insert column into collection tube and load upto 600 Î¼l sample into the column. Spin for 1 minute at 16,000 x g or at maximum speed in micro centrifuge. Discard the flow-through. Repeat this step if volume is more than 600 Î¼l.
5. Re-insert column into collection Add 250 Î¼l of DNA Wash Buffer and spin for 1 minute at maximum

6. Transfer column to a clean 5 ml microfuge tube and spin for 1 minute to ensure the removal of traces of salt and ethanol, so that they are not carried over to next step. Discard the microfuge tube and save the column for next step
7. Place the Column in a clean 5 ml microfuge tube and add 10â€“20 Î¼l of DNA Elution Buffer to the center of the column. In order to obtain maximum elution, make sure the Elution buffer is in direct contact with membrane in the center rather than sticking to the wall of the column.
8. Wait for 1 minute, and then spin at maximum speed for 1 minute to elute

Date Created
2024/07/03