GeneJET PCR Purification Kit, K0701

Introduction

This protocol is for PCR (DNA) purification with the Thermo Scientific GeneJET PCR Purification Kit K0701. The protocol is based on the user guide found on the following website: https://www.thermofisher.com/order/catalog/product/K0701#/K0701

Note: Read IMPORTANT NOTES on p. 3 before starting.

- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out in a table-top microcentrifuge at >12000 x g (10 000-14 000 rpm, depending on the rotor type).

Materials

- Kit contains:
  - Binding Buffer
  - Wash Buffer
  - Elution Buffer
  - Nuclease-free H20

Procedure

1. DNA Purification using centrifuge
2. Add a 1:1 volume of Binding Buffer to the completed PCR mixture (e.g. for every 100µLof reaction mixture, add 100 µLof Binding Buffer). Mix thoroughly. Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µLof 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
3. Optional: if the DNA fragment is ≤500bp, add a 1:2 volume of 100% isopropanol (e.g.,100 µLof isopropanol should be added to 100 µLof PCR mixture combined with 100 µLof Binding Buffer). Mix thoroughly.

   Note: If PCR mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower.

4. Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the GeneJET purification column. Centrifuge for 1-2 min. Discard the flow-through. Notes. If the total volume exceeds 800 µL, the solution can be added to the column in stages. After the addition of 800 µL of solution, centrifuge the column for 1-2 min and discard flow-through. Repeat until the entire

solution has been added to the column membrane. Close the bag with GeneJET Purification Columns tightly after each use!

5. Add 700 µL of Wash Buffer (diluted with the ethanol as described on p. 3) to the GeneJET purification column. Incubate for 1-2 min. Centrifuge for 1-2 min. Discard the flow-through and place the purification column back into the collection tube.

   Note: Wash buffer can be added in two turns with incubation and centrifugation in between.

6. Centrifuge the empty GeneJET purification column for an additional 1-5 min to completely remove any residual wash buffer.

   Note. This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.

7. Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube (not included). Add 15-50 µL of Elution Buffer to the center of the GeneJET purification column membrane and centrifuge for 1-2 min.

   Make sure you drop the elution buffer exactly on the filter, without touching it with the pipette tip. After that, incubate at least 1 minute at room temperature, and then centrifuge. This step is key for efficient elution, failing to do this will significantly reduce the yield of DNA.

   Note: For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended.

   If the DNA fragment is >10 kb, prewarm Elution Buffer to 65 °C before applying it to column.

   If the elution volume is 10 µL and DNA amount is ≥5 µg, incubate column for 1 min at room temperature before centrifugation.

8. Discard the GeneJET purification column and store the purified DNA at -20 °C.