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DNA Purification from a PCR Product (Protocol for NucleoSpin® PCR clean-up Gel Extraction Kit)

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We use this protocol and it's working

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Abstract

The following protocol is suitable for PCR clean-up as well as DNA concentration and removal of salts, enzymes, etc. from enzymatic reactions (SDS < 0.1 %).

Materials

MATERIALS

GeneJET Gel Extraction and DNA Cleanup Micro Kit **Thermo Fisher Catalog #**K0832

Troubleshooting

Safety warnings

Buffer NTI contains chaotropic salt. Wear gloves and goggles. Buffer NTI contains guanidinium thiocyanate which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Always wear gloves and goggles and follow the safety instructions given in the kit.

Before start

Check if Wash Buffer NT3 was prepared according to the information given in the kit.

For Wash Buffer NT3: Add the indicated volume of ethanol (96–100 %) to Buffer NT3 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Wash Buffer NT3 is stable at room temperature (18–25ºC) for at least one year.

1 Adjust DNA binding condition, For very small sample volumes < 30 µL adjust the volume of the reaction mixture to 50–100 µL with water. It is not necessary to remove mineral oil. Mix 1 volume of sample with 2 volumes of Buffer NTI (e.g., mix 100 µL PCR reaction and 200 µL Buffer NTI).

'Note': For removal of small fragments like primer dimers dilutions of Buffer NTI can be used instead of 100 % Buffer NTI. Incubate the mixture at 50ºC for 5-10 minutes or until the gel has completely melted.

2 Place a NucleoSpin® PCR clean-up Gel column in a provided 2 mL collection tube. Apply up to 700 µL of the DNA/agarose solution to the NucleoSpin® PCR cleanup Gel column, and centrifuge at 11, 000 x g for 30 seconds at room temperature.

3 Discard liquid and place the NucleoSpin® PCR clean-up Gel column back into the same collection tube. For volumes greater than 700 µL, load the column and centrifuge successively, 700 µL at a time. Each NucleoSpin® PCR clean-up Gel column has a total capacity of 25 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

4 Add 700 µL of Buffer NT3 into the NucleoSpin® PCR clean-up Gel columnCentrifuge at 11,000 x g for 30 seconds at room temperature to wash the column. Discard the flow-through and re-use the collection tube.

5 Repeat step 4 with another 700 µL of Buffer NT3.

6 Discard liquid and centrifuge the empty NucleoSpin® PCR clean-up Gel column for 1 minute at 11,000 x g to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the NucleoSpin® PCR clean-up Gel column.
'Note': Residual ethanol from Buffer NT3 might inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2–5 minutes at 70ºC prior to elution.

7 Place a NucleoSpin® PCR clean-up Gel column into a clean 1.5 mL eppendorf tube. Add 15-30 µL (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix and incubate at room temperature for 1 minute. Centrifuge for 1 minute at 11,000 x g to elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

'Note': DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with fresh buffer, heating to 70ºC and incubation for 5 minutes.