PCR Purification Protocol

Modified from GeneJET PCR Purification Kit

Preparation:

Dilute Wash Buffer (concentrated) by adding 5:1 volume of ethanol (96-100%).

Procedure:

All centrifugations should be carried out in a tabletop microcentrifuge at >12000×g (10 000-14 000 rpm).

1. Add a 1:1 volume of Binding Buffer to completed PCR mixture. 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 µL of 3 M sodium acetate solutions (pH 5,2) and mix. The color of the mix will become yellow.

2. If the DNA fragment is ≤500bp, add a 1:2 volume of 100% isopropanol. Mix thoroughly.

3. Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through.

4. Add 700 µL of wash Buffer (diluted with the ethanol) to the GeneJET purification column. Centrifuge for 30-60 s. Discard the flow-through and place the purification column back into the collection tube.

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

6. Transfer the GeneJET purification column to a clean 1.5 mL microcentrifuge tube. Add 50 µL of water to the center of the GeneJET purification column membrane and centrifuge for 1 min.

   Note. For low DNA amounts the elution volumes can be reduced to increase DNA concentration. Elution volumes less than 10 µL are not recommended.

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