Protocol: PCR Purification Protocol

QIAquick PCR Purification Kit (50)

All centrifugation steps are carried out at 13,000 rpm in a conventional table-top microcentrifuge at room temperature.

1. Add 5 volumes Buffer PB to 1 volume of PCR reaction and mix. If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The colour of the mixture will turn yellow.
2. Place a QIAquick column in a provided 2 ml collection tube.
3. To bind DNA, apple the sample to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QIAquick column back in the same tube.
4. To wash, add 0.75 ml buffer PE to the QIAquick column and centrifuge for 1 min. Discard flow-through and place the QIAquick column back in the same tube.
5. Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
6. Place each QIAquick column in a clean 1.5 ml microcentrifuge tube.
7. To elute DNA, add 50 µl of distilled water (pH 7.0 to 8.5) or buffer EB to the centre of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30 µl Buffer EB to the centre of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.
8. If the purified DNA is to be analysed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

Buffers:

- Buffer PB - Binding buffer (has pH indicator added)
- Buffer PE - Wash buffer (has ethanol added)
- Buffer EB - Elution buffer

Note: Measure DNA concentration using NanoDrop Spectrophotometer before running any digestion