PCR Purification Kit

Cat#: orb533436 (User Manual)

Description:
PCR Purification Kit is designed for the work-up of PCR reactions (removal of primer dimers, primers, nucleotides, proteins, salt, agarose, ethidium bromide, and other impurities). The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. The kit provides a simple and efficient way to purify linear or circular DNA in the size range from 100 bp to 10 kb and is optimized for working with DNA amounts of up to 20 µg. It requires no organic extractions or precipitation and guarantees high and stable recovery rates.

Content:
Binding Buffer
Activation Buffer
Washing Buffer (before use, add 96-99 % Ethanol as indicated on the bottle)
Elution Buffer
Spin Columns
2 ml Collection Tubes

To be provided by you:
96-99 % Ethanol
Isopropanol (for high yield sample preparation)
1.5 ml microtubes

Preparation procedure:
The DNA purification follows a simple binding, washing and eluting procedure. Before start, add 96-99 % Ethanol to the Washing Buffer as indicated on the bottle.
The additional use of Isopropanol is recommended for fragments smaller than 200 bp or larger than 5 kbp.
The optional secondary washing step minimizes the salt content of the purification product but may significantly reduce the yield of DNA fragments <200 bp.

1a Standard Sample Preparation:
For DNA fragment sizes in the range of 200 bp to 5 kbp:
• Add 5 volumes of Binding Buffer to 1 volume of DNA sample and mix well. For example, if the volume of your DNA sample is 50 µl, add 250 µl Binding Buffer.

1b High Yield Sample Preparation:
For DNA fragment sizes smaller than 200 bp or larger than 5 kbp:

• Add 3 volumes Binding Buffer and 2 volumes of Isopropanol to the PCR sample. For example, if the volume of your DNA sample is 50 µl, add 150 µl Binding Buffer and 100 µl Isopropanol.

2 Column Activation:
• Place a Spin Column into a 2 ml collection tube
• Add 100 µl of Activation Buffer into the Spin Column.
• Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.

3 Column Loading:
• Apply the sample mixture from step 1 into the activated Spin Column.
• Centrifuge at 10,000 g for 30 sec in a micro-centrifuge.
• Discard the flow-through.

4 Column Washing:
• Place the DNA loaded Spin Colum into the used 2 ml tube.
• Apply 700 µl of Washing Buffer to the Spin Column.
• Centrifuge at 10,000 g for 30 sec and discard the flow-through.
Optional Secondary Washing: Recommended only for DNA >200 bp, if highly purified DNA (for DNA sequencing, transfection etc.) is required.
• Add 700 µl of Washing Buffer to the Spin Column.
• Centrifuge at 10,000 g for 30 sec and discard the flow-through.
• Centrifuge again for 2 min to remove residual Washing Buffer.

5 Elution:
• Place the Spin Column into a clean 1.5 ml microtube (not provided in the kit).
• Add 30-50 µl Elution Buffer or dd-water to the center of the column membrane.
• Incubate at room temperature for 1 min.
• Centrifuge at 10,000 g for 1 min to elute DNA.