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PCR and Gel Extraction Mini Prep Kit

Kit for the purification of DNA from PCR reactions AND for extraction of DNA fragments from standard or low-melt agarose gels

Cat#: S5380

Protocol only

Version: 220410

PCR DNA Purification Protocol

This protocol is designed for purification of single- or double stranded DNA fragments **(100bp up to 10kbp)** from up to 200µL PCR-samples.

1. **Add 2 volumes** of **CB Buffer** to 1 volume **of DNA containing sample** (for example add 100µL CB buffer to a 50µL PCR reaction) and vortex for 3 sec..
   NOTE: The solution should be yellow. If it turns pink after mixing, add 10µL of 3M sodium acetate, pH5.2, and mix thoroughly.
   NOTE: For sample preparation method, see instructions given on page 3 and 4.
2. Centrifuge the tube briefly in order to recover any remaining liquid from the lid and transfer the whole volume of the mixture into a DNA purification column placed in a collection tube (2mL).
3. Centrifuge for **30 seconds at 11,000k x g**. Discard the filtrate.
4. Transfer the purification column in a provided 2mL collection tube.
5. Add **700µL Wash Buffer** (add ethanol to Wash Buffer before use) to the column and centrifuge for **30 sec. at 11,000k x g**
6. Discard flow-through and place the column back in the same tube.
7. Recommended: repeat previous washing step (Optional but recommended step).
   Add **500µL Wash Buffer** (add ethanol to Wash Buffer before use) to the column and centrifuge for **30 sec. at 11,000k x g**
8. Discard the flow-through and re-use collection tube.
9. Centrifuge for **60 seconds at 11,000k x g**
   NOTE: The wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease elution efficiency. It is therefore vital to remove the alcohol completely from the column before elution.
10. Discard the collection tube with the flow-through and carefully transfer the purification column to a new sterile 1.5mL micro centrifuge tube.
11. Add **15µL-30µL Elution Buffer** directly onto the purification column membrane (pipette liquid in the inner part of the blue ring).
12. Incubate the column at room temperature for 60 seconds.
13. Centrifuge for 6**0 seconds at 11,000k x g**
14. Remove the mini column.
    The isolated DNA is ready for use in downstream applications or for short-term storage at +2°C to +8°C or for long-term storage at -20°C.

Gel extraction DNA Purification Protocol

This protocol is designed for the extraction and purification of DNA from up to 300mg standard or low-melt agarose gel per spin.

1. Excise a gel slice containing the DNA fragment and place it in a 1.5-2mL micro centrifuge tube.
   NOTE: The gel slice mass should not exceed 300mg. For instructions, see page 4.
2. Add **400µL GB Buffer** and mix well by inverting the tube several times.
3. Incubate the mixture at 50°C for 5-10 min (or until the gel slice has completely dissolved). During incubation mix the sample by inverting the tube several times.
   NOTE: Ensure that the agarose is completely dissolved before moving on to the next step.
   NOTE: The solution should be yellow. If it turns pink after mixing, add 10µL of 3M sodium acetate, pH5.2 and mix thoroughly (see page 3).
4. Add **250µL isopropanol** (not included in this kit) and mix well by inverting the tube several times.
5. Centrifuge the tube briefly to recover any remaining liquid from the lid and transfer **800µL** of the mixture into a DNA purification column placed in a collection tube (2mL).
   Centrifuge for **30 seconds at 11,000k x g**
   NOTE: IF the volume of the mixture exceeds 800µL in total, discard the flow-through after centrifugation, then reuse the collection tube and transfer the remaining mixture into the same mini column.
6. Transfer the purification column to a new 2mL collection tube.
7. Add **700µL Wash Buffer** (add ethanol to Wash Buffer before use) to the column and centrifuge for **30 sec. at 11,000k x g**
   Discard flow-through and place the column back in the same tube.
8. Recommended: repeat previous washing step (Optional but recommended step).
   Add 5**00µL Wash Buffer** (add ethanol to Wash Buffer before use) to the column and centrifuge for **30 sec. at 11,000k x g**
   Discard flow-through and place the column back in the same tube.
9. Centrifuge for **60 seconds at 11,000k x g**
   NOTE: The wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease elution efficiency. It is therefore vital to remove the alcohol completely from the column before elution.
10. Carefully transfer the purification column to a sterile, 1.5mL microcentrifuge tube.
11. Add **50µL Elution Buffer** directly onto the purification column membrane.
    NOTE: Other buffer volumes in the 20 – 200µL range may be used. For instructions, see page 3.
12. Incubate the mini column at room temperature for 120 seconds.
13. Centrifuge for **60 seconds at 11,000k x g**
14. Remove the mini column. The isolated DNA is ready for use in downstream applications or for short-term storage at +2°C to +8°C or for long-term storage at -20°C