DNA product purification kit

Project number: D665967

Storage conditions: room temperature.

Product Content:

Component	D665967 50preps	D665967 200preps
BufferPB	30ml	120ml
BufferPS	15ml	60ml
BufferPW (concentrate)	6ml	25ml
BufferEB	10ml	30ml
SpinColumnsDM withCollectionTubes	50	200

Product Description:

This kit adopts the new silicon matrix membrane technology and reagent formulation, which can purify and recover DNA fragments of 100bp-10kb from PCR products or enzyme reaction solutions (digestion, ligation, probe labeling, etc.) through three quick and simple binding-washing-elution steps, and each adsorption column can adsorb up to 10 μg of DNA, while maximally removing the primers, oligonucleotides, enzymes and other impurities. The purified and recovered DNA has high purity and concentration, good integrity and high recovery rate, and can be directly used in molecular biology experiments such as sequencing, ligation and transformation, labeling and in vitro transcription.

Self-contained reagent: anhydrous ethanol.

Lab prep and important notes:

1. All components can be stored stably in a dry, room temperature (15-30℃) environment for 1 year, and can be stored at 2-8℃ for longer periods of time. When the solution is stored at low temperature, it is necessary to leave it at room temperature for a period of time before use, and then use it after restoring it to room temperature.
2. This kit can non-selectively recover all DNA fragments in solution. If you need to selectively recover specific fragments and remove other fragments of different sizes at the same time, please choose our gel recovery kit.
3. Anhydrous ethanol should be added to BufferPW according to the instructions on the label of the reagent bottle before first use.

4. Before use, please check whether BufferPB is crystallized or precipitated, if there is any crystallization or precipitation phenomenon, it can be restored to clarity in a 37℃ water bath for a few minutes.
5. The recovery efficiency is related to the initial amount of DNA and the elution volume; the lower the initial amount and the lower the elution volume, the lower the recovery rate.
6. All centrifugation steps can be performed at room temperature.

Operational Steps:

1. Estimate the volume of the DNA reaction solution, add 5 times the volume of BufferPB and mix well (no need to remove paraffin oil or mineral oil).

Note: 1) If the DNA reaction system is 50μl (excluding paraffin oil volume), add 250μl BufferPB.

2) Test the pH of the solution after adding BufferPB, if the pH is > 7.5, add 10-30 μl of 3M sodium acetate (pH 5.0) to it, thus adjusting the pH to 5-7.

2. Column equilibration: Add 200 μl BufferPS to the adsorbent column (SpinColumnsDM) that has been loaded into the collection tube, centrifuge at 13,000 rpm (~16,200 × g) for 1 minute, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.

3. Add the solution obtained in step 1 to the adsorption column which has been loaded into the collection tube, leave it at room temperature for 1 minute, centrifuge it at 13,000 rpm for 30-60 s, pour out the waste liquid in the collection tube and put the adsorption column back into the collection tube.

Note: The adsorption column volume is 750μl, if the sample volume is larger than 750μl, it can be added in batches.

4. Add 500μl BufferPW to the adsorption column (check that anhydrous ethanol has been added before use) Centrifuge the column at 13,000rpm for 30-60s, pour off the waste liquid from the collection tube and place the column back into the collection tube.

Note: If purified DNA is used for salt-sensitive experiments (e.g., flat-end ligation experiments or direct sequencing), it is recommended that it be left to stand for 2-5 minutes before centrifugation after adding BufferPW.

5. Centrifuge at 13,000 rpm for 1 minute and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorbent column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.). To ensure that downstream experiments are not affected by

residual ethanol, it is recommended that the adsorbent column be uncapped and left at room temperature for a few minutes to thoroughly dry the residual ethanol in the adsorbent material.

6. Place the adsorbent column in a new centrifuge tube (supplied), add 30-50μl of BufferEB to the center of the adsorbent membrane overhang, and allow to stand at room temperature for 1 min. centrifuge at 13,000 rpm for 1 min. to collect the DNA solution. Store the DNA at -20° C.

Note: 1) The pH of the eluent has a great influence on the elution efficiency. If water is used as the eluent, its pH should be ensured to be between 7.0 and 8.5 (the pH of water can be adjusted to this range with NaOH).

2) To increase the amount of DNA recovered, the solution obtained by centrifugation can be re-added to the adsorption column, left at room temperature for 2 minutes and centrifuged at 13,000 rpm for 1 minute.

3) The elution volume should not be less than 30 μl, too little volume will affect the recovery efficiency.

4) When recovering DNA fragments >10kb, BufferEB should be preheated in a 50° C water bath, and the appropriate extension of adsorption and elution time can increase the recovery efficiency.