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Sequencing PCR product-Clean up Kit

Product Number: SGS38

Shipping and Storage

1. Store at 2-8°C for a maximum of 12-18 months;
2. Mix thoroughly before use to avoid sedimentation and clumping; Wait until the solution returns to room temperature to avoid volume changes caused by temperature differences;
3. Cannot be frozen, freezing will damage the structure of magnetic beads;

Components

Component	SGS38
Sequencing PCR product-Clean up Kit	50ml

Description

The Sequency PCR product Clean up system uses special chemical reactions and reversible "nucleic acid magnetic bead" binding technology to deliver and reliably purify PCR amplification products on a large scale. By optimizing the buffer solution, magnetic beads selectively bind to amplification fragments of over 100bp. The remaining primers, nucleic acid templates, salt ions, enzymes, primer dimers, and some non-specific amplification small fragments in PCR reactions can be completely removed by washing, thus obtaining ideal target fragments. The purified product can be applied in molecular biology applications such as PCR, sequencing (including first generation Sanger sequencing and second-generation sequencing), genotyping, fragment analysis, gene cloning, etc.

Experimental preparation

1. 96 well plate purification: 96 well PCR plate (can be used for reaction systems less than 50μl);
   96 well microplate (volume must be greater than 300μl, used for reaction systems greater than 50μl, such as 100μl reaction systems)
2. 384 well plate purification: 384 well plate PCR plate (generally used for reaction systems with a volume less than 10μl, containing 10μl; for purification of reaction systems larger than 10μl, it is recommended to use a larger volume 384 well plate for purification to better avoid cross contamination)
3. Magnetic frame (divided into magnetic frames for 96 well plates and 384 well plates); Vortex oscillator; Adjustable multi-channel pipette; Liquid storage tank; Sealing film; 96 well microporous plate;
4. 70% ethanol (note: 70% ethanol has water absorption, so using newly formulated 70% ethanol for purification is more effective. If using old 70% ethanol, it is recommended to use it under sealed conditions, and the usage period of 70% ethanol should not exceed one week)
5. Buffer EB
   Note: Buffer EB can be used with 10 mM Tris pH 8.0, deionized sterilized ultrapure water, or TE Buffer (10 mM Tris pH 8.0, 1mM EDTA)

Protocol

96 well plate operation standard process

1. **Carefully read the operation manual and determine whether to perform plate transfer and purification based on the size of the reaction system.**
   If the total volume of the PCR reaction is multiplied by 2.8 and the final total volume exceeds the effective volume of the PCR plate, plate transfer is required. Depending on the size of the final volume, a microplate with a volume of 300μl or larger can be selected for purification.

2. **Shake the Sequencing PCR product Clean up reagent bottle and resuspend the magnetic beads inside. Add magnetic bead solution of corresponding volume size to the PCR product according to the following volume ratio.**

The magnetic bead solution must be shaken thoroughly before use to avoid precipitation and clumping, and the solution should be returned to room temperature.

Volume of PCR reaction solution (μl)	Volume of Sequencing PCR product-Clean up (μl)
10	18
20	36
50	90
100	180

Note:1) The calculation method for the volume of magnetic bead solution is: the amount of magnetic bead solution=1.8 x the reaction volume of PCR product;

2)Conventional PCR purification involves removing primer dimers, fragments smaller than 100bp, etc., in a 1:1 ratio.

3. **Blow and mix 10 times with a pipette or vortex for 30 seconds, thoroughly mix the magnetic beads and PCR products, and let them stand at room temperature for 5 minutes.**

This step is to allow the magnetic beads to bind and adsorb fragments of over 100bp (including 100bp). It is recommended to use a pipette to blow and mix well, as the results are more reproducible. At the same time, the color of the solution should be uniform after mixing, and there should be no local enrichment of magnetic beads.

4. **Place the PCR plate on the magnetic rack and let it stand for two minutes. The magnetic beads will adsorb and accumulate at a certain point at the bottom or edge of the PCR plate, and the solution will become clear.** Before proceeding to the next experiment, the solution here must become clear. If it is not clear, it can be left to stand for a few more minutes (depending on the magnitude of the magnetic force on the magnetic frame).

5. **Absorb the supernatant and discard it without touching the magnetic beads.**

This step requires the 96 well plate to be fixed on the magnetic frame and not to touch the magnetic beads. If some of the magnetic beads are resuspended due to excessive suction of the pipette, a small amount of supernatant can be left inside.

6. **Add 100μl of 70% ethanol to each well, incubate at room temperature for 30 seconds, then aspirate and discard the added ethanol, repeat twice.**

This step requires the 96 well plate to be fixed on the magnetic bracket and not to touch the magnetic beads. The last time, the residual ethanol at the bottom must be completely removed. If necessary, it can be gently flipped and shaken a few times, and then dried at room temperature for 5-8 minutes or placed in an oven at 60°C for 2-3 minutes. Because residual ethanol is an inhibitor of PCR.

Note: The drying time for this step should not be too long. Excessive drying can damage the structure of the magnetic bead surface, thereby affecting the efficiency of purification and recovery.

7. **Remove the purified PCR plate from the magnetic rack, add 25μl Buffer EB to each well, and mix 10 times with a pipette or vortex shake for 30 seconds.**

The elution volume of 25μl here is generally sufficient for subsequent experiments. If a larger volume is required, it is also possible, but if the elution volume is less than 25μl, it is necessary to ensure that all magnetic beads can repeatedly come into contact with the Buffer EB, otherwise it will affect the efficiency of elution. The simultaneous elution speed is very fast, as long as the magnetic beads come into contact with the Buffer EB, the PCR product can dissolve into the Buffer EB.

8. **Reposition the 96 well purification plate onto the magnetic rack and let it stand at room temperature for 1-2 minutes to allow the magnetic beads to fully adsorb and enrich to a point at the bottom or edge, and the solution becomes clear.**

9. **Transfer the supernatant onto a new PCR plate.** It is recommended to freeze the purified PCR products for long-term storage. After the transfer, it can avoid the breakage of magnetic beads.

Standard Operating Procedure for 384 well Plate

1. **Carefully read the operation manual and decide whether to perform plate transfer and purification based on the size of**

the reaction system.

If the total volume of the PCR reaction is multiplied by 2.8 and the final total volume exceeds the effective volume of the PCR plate, plate transfer is required. The maximum reaction system that can be purified by a regular 384 well PCR plate is 14μl. If it exceeds this limit, it needs to be transferred to a larger volume 384 well plate or split into 96 well PCR plates for purification

2. **Gently shake the Sequencing PCR product Clean up reagent bottle and resuspend the magnetic beads inside. Add the corresponding volume of magnetic bead solution to the PCR product according to the following volume ratio.** The magnetic bead solution must be shaken thoroughly before use to avoid precipitation and clumping.

Volume of PCR reaction solution (μl)	Volume of Sequencing PCR product-Clean up (μl)
5	9
7	12.6
10	18
14	25

Note: 1) The calculation method for the volume of magnetic bead solution is: the amount of magnetic bead solution=1.8 x the reaction volume of PCR product;

2) Due to the volume limitation of the 384 well PCR plate, the maximum volume that a regular 384 well PCR plate can purify is 14μl of the reaction system.

3) Conventional PCR purification involves removing primer dimers, fragments smaller than 100bp, etc., in a 1:1 ratio.

3. **Blow and mix 15 times with a pipette, thoroughly mix the magnetic beads and PCR products, and leave at room temperature for 5 minutes.**

This step is to allow the magnetic beads to bind to fragments of over 100bp (including 100bp). It is recommended to use a pipette to blow and mix well, as the results are more reproducible. At the same time, the color of the solution should be uniform after mixing, and there should be no local magnetic bead enrichment phenomenon.

4. **Place the 384 well PCR plate on a magnetic rack and let it stand for two minutes. The magnetic beads will adsorb and accumulate at a certain point at the bottom or edge of the PCR plate, and the solution will become clear.** Before proceeding to the next experiment, the solution here must become clear. If it is not clear, it can be left to stand for a few more minutes (depending on the magnitude of the magnetic force on the magnetic frame).

5. **Absorb the supernatant and discard it, without touching the magnetic beads**

This step requires the 384 well plate to be fixed on the magnetic frame and not to touch the magnetic beads. If some of the magnetic beads are resuspended due to excessive suction of the pipette, a small amount of supernatant can be left inside.

6. **Add 30μl of 70% ethanol to each well, incubate at room temperature for 30 seconds, then aspirate and discard the added ethanol, repeat twice.**

This step requires the 384 well plate to be fixed on the magnetic bracket and not to touch the magnetic beads. The last time, the residual ethanol at the bottom must be completely removed. If necessary, it can be gently flipped and shaken a few times, and then dried at room temperature for 5-8 minutes or placed in an oven at 60°C for 2-3 minutes. Because residual ethanol is an inhibitor of PCR.

Note: The drying time for this step should not be too long. Excessive drying can damage the structure of the magnetic bead surface, thereby affecting the efficiency of purification and recovery.

7. **Remove the purified 384 well PCR plate from the magnetic rack, add 20μl Buffer EB to each well, and mix 10 times with a pipette or vortex shake for 30 seconds.**

The elution volume of 20μl here is generally sufficient for subsequent experiments. If a larger volume is required, it is also possible, but if the elution volume is less than 15μl, it is necessary to ensure that all magnetic beads can repeatedly come into contact with the Buffer EB, otherwise it will affect the efficiency of elution. The simultaneous elution speed is very fast, as long as the magnetic beads come into contact with Buffer EB, the PCR product can dissolve into the Buffer EB.

8. **Reposition the 384 PCR purification plate onto the magnetic rack and let it stand at room temperature for 1-2 minutes to allow the magnetic beads to fully adsorb and enrich to a point at the bottom or edge, and the solution becomes clear.**

9. **Transfer the supernatant onto a new PCR plate.**

It is recommended to freeze the purified PCR products for long-term storage. After the transfer, it can avoid the breakage of magnetic beads.