Tinzyme Co., Limited

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Big Dye Purification kit

Product Number: SGS36

Shipping and Storage

1. For long-term storage of magnetic beads, please store them at 2-8°C. After each use, please place them in a refrigerator at 4°C and do not stick them to the innermost wall of the refrigerator, as the temperature on the innermost wall of domestic refrigerators can reach 0°C or even lower, and they will freeze over time. This magnetic bead product is strictly prohibited from freezing.
2. Both large and small packages must be thoroughly mixed before each use;
3. For long-term storage, it is recommended to cover it with a black plastic bag and store it away from light.

Description

This kit can specifically bind to all fragments in Sanger sequencing reaction through optimized magnetic bead system, but not to dNTPs, ddNTPs, enzymes and other raw materials. Magnetic beads adsorb all fragments generated by one-way PCR amplification under a certain proportion of ethanol conditions, and then wash them with 85% ethanol to remove all remaining raw materials from the reaction, thereby achieving purification. The purified product can be directly subjected to electrophoresis sequencing using a genetic analyzer such as 3730xl. The signal is strong and uniform, with extremely low background noise. It can completely replace the traditional EDTA/ethanol precipitation method, facilitate automated operation, and reduce human errors.

Experimental preparation

1. 96 well PCR plate or 384 well PCR plate
2. Pipette and tip (100μl, 10μl)
3. Disposable gloves or latex gloves
4. Vortex oscillator
5. Anhydrous ethanol
6. Magnetic holder, etc

Note:85% ethanol (note: 85% ethanol has water absorption, so using newly prepared 85% ethanol for purification is more effective. If using old 85% ethanol, it is recommended to use it under sealed conditions, and the usage period of 85% ethanol should not exceed one week)

Protocol

96 well plate operation standard process

1. Remove the magnetic beads from the 4 ℃ refrigerator and mix thoroughly.

   Note:1).After closing the lid tightly, the magnetic beads must be fully resuspended and there should be no clumping of magnetic beads;

   2).After use, put it back in the refrigerator at 2-8°C for storage.
2. Remove the 96 well PCR plate that has completed the reaction from the PCR instrument, centrifuge it instantly, and centrifuge the droplets on the tube wall to the bottom of the tube.
3. Add 5μl of magnetic beads and 21μl of 85% ethanol (for sealing purposes) to a 5μl PCR reaction system, shake and mix after sealing (it is recommended to shake and mix for at least 30 seconds), or use a pipette to blow and mix (at least 10 times). If it is another PCR reaction system, you can refer to the following table for sample addition:

System	85% anhydrous ethanol dosage
5μl	21μl
10μl	42μl

Note: 1).85% ethanol must be registered and can be measured using an alcohol meter. Because different concentrations

of anhydrous ethanol and measuring cylinders have the same accuracy.

2).After using 85% anhydrous ethanol, the lid should be tightly closed in a timely manner to avoid the volatilization of high concentration ethanol. It is recommended that the ethanol prepared each time should not exceed 7 days.

4. Let it stand for 2 minutes, mix 1-2 times during this period, then place the PCR plate on a magnetic rack and adsorb for 2 minutes to remove the supernatant.

   Note: During the static period, it is necessary to mix thoroughly 1-2 times. If it is a machine automated blowing and mixing process, blowing and mixing 8-10 times is sufficient; If it is manual oscillation mixing, it is recommended to oscillate once every 30 seconds and mix twice.

5. Keep the PCR plate on the magnetic rack, add 100μl of 85% ethanol, incubate at room temperature for 1-2 minutes, and then directly remove the supernatant;

   Note: Carefully observe whether there are bubbles at the bottom of the hole, and ensure that all solutions reach the bottom of the tube.

6. Repeat step 5 once, and finally keep the PCR plate on the magnetic rack. Cover the plate holes with absorbent paper and try to shake off the liquid droplets in the holes as much as possible, or use a pipette to absorb the remaining alcohol droplets.

7. Keep the PCR plate on the magnetic rack, invert it in an oven at 60 ℃ for 2 minutes, and stand it upright for 3 minutes to allow ethanol to evaporate completely; Or let it stand at room temperature for 10 minutes to allow the ethanol to evaporate completely.

   Note: This step must ensure that ethanol evaporates completely, otherwise interference peaks may occur later.

8. Add 25μl~50μl of deionized sterile water to seal the film and shake well to dissolve and disperse the magnetic beads. For individual magnetic bead samples that are difficult to disperse, use a 20μl pipette to repeatedly aspirate and mix the magnetic beads until they are fully mixed. After instantaneous centrifugation, let it stand at room temperature for 2 minutes;

   Note: It must be deionized sterile water. If it contains electric ions, the subsequent electrophoresis signal value will decrease to no sequencing signal based on the amount of electric ions.

9. Adsorb on the magnetic rack for 2 minutes, carefully transfer 10μl of the supernatant onto a new 96 well plate using a 10μl pipette, avoid inhaling magnetic beads, and perform electrophoresis sequencing on the machine.

10. The remaining sample plates with magnetic beads should be stored in the refrigerator.