【Product Name】CleanSeq Beads

【Product specifications】5ml, 50ml, 500ml

【Intended Use】

A rapid, high performance dye-terminator removal process based on theparamagnetic bead technology. The paramagnetic bead format requires no centrifugation or filtration and is easily performed manually or fully automated for high throughput dye-terminator removal. Compared to similar systems,This product produces sequences with longer Phred 20 read lengths and higher signal intensities than any other purification technology.

【Principle】

The CleanSEQ method contains magnetic particles in an optimized binding buffer to selectively capture sequencing extension products. The protocol can be performed directly in the thermal cycling plate. Unincorporated dyes, nucleotides, salts and contaminants are removed using a simple washing procedure. The purification procedure is amenable to a variety of automation platforms since it requires no centrifugation or vacuum filtration.

【Main Composition】

Cat.No.	BCS-5	BCS-50	BCS-500
CleanSeq Beads	5 ml	50 ml	500 ml

【Storage conditions and Validity】

CleanSeq Beads should be stored at 2–8°C upon arrival and is stable up to 6 months under the condition. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect its performance. Mix CleanSEQ Beads well before using. The reagent should appear homogenous and consistent in color.

DO NOT FREEZE.

【Preparation before Use】

- Elution Buffer: Reagent grade water, 0.1 mM EDTA (pH 8.0), or 0.5 mM EDTA (pH 8.0). The optimal elution buffer will vary depending on dye chemistry and reaction conditions.
- magnetic plate
- For 96 well format: Fresh 85% Ethanol made with Non-denatured Ethanol.

【Protocol】

1. Gently shake the CleanSeq Beads bottle to resuspend any magnetic particles that may have settled. Add 5μL of CleanSeq Beads to the reaction plate.
   This step should be performed before the plate is placed on the SPRIPlate 96R ring magnetic plate. Use 5 μL of CleanSeq Beads regardless of the sequencing reaction volume.
2. Add 85 % ethanol to the reaction plate according to the table below. Pipette mix 10 times or shaking the reaction plate for 30 seconds.
   This step should also be performed before placing the plate on the magnetic plate. If you are vortexing, use a medium speed and make sure the suspension is completely homogeneous before continuing. The volume of 85% ethanol needed has been calculated for common sequencing reaction volumes, as shown in Table.

Volume of Sequencing Reaction (μL)	Volume of CleanSeq Beads	Volume of 85% Ethanol
5μL	5μL	47μL
10μL	5μL	70μL
15μL	5μL	93μL
20μL	5μL	117μL

3. Incubate the reaction plate for at least 3 minutes at room temperature.
   This incubation must be performed before the reaction plate is placed on the magnetic plate.
4. Place the reaction plate onto the magnetic plate for 3 minutes to separate beads from solution.
   The solution should be clear before proceeding to the next step.
5. Aspirate the cleared supernatant from the reaction plate and discard.
   This step should be performed while the plate is situated on the magnetic plate. Be careful not to disturb the beads. It is important to completely remove all of the supernatant, as it contains excess fluorescent dye and contaminants.

6. Dispense 100 μL of 85 % ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard.
   This step should be performed while the plate is situated on the magnetic plate. There is no need to agitate the beads.

7. Dispense 100 μL of 85 % ethanol and incubate at room temperature for at least 30 seconds. Aspirate out the ethanol and discard.
   This step should be performed while the plate is situated on the magnetic plate. There is no need to agitate the beads. It is important to remove all of the ethanol, as it contains residual fluorescent dye and contaminants.

8. Let the reaction air-dry for 10 minutes at room temperature.
   The reaction plate does not have to be situated on an magnetic plate during drying. Note that excessive drying can lead to degradation of incorporated dye terminators. After removing the final ethanol wash, allow the samples to dry at room temperature for approximately 10 minutes. The plate may then be sealed and stored dry at 4°C or -20°C. Elute the samples just prior to loading on the sequencing detector.

9. Add 30 μL of elution buffer and incubate the plate for 5 minutes at room temperature to elute.
   The elution of sequencing products from the magnetic beads is rapid, and it is not necessary for the beads to go back into solution for complete recovery of the samples. Suggested elution buffers are reagent grade water (optimal), 0.1 mM EDTA (pH 8.0), or 0.5 mM EDTA (pH 8.0). For maximum signal intensity, elute samples in reagent grade water. If overloading of sample occurs, using 0.1 mM EDTA as an elution buffer can help to temper the signal and improve readlengths and Phred 20 scores. If overloads are still seen with 0.1 mM EDTA, use 0.5 mM EDTA instead. An increase in the concentration of EDTA will decrease the signal strength.

   Note: Formamide can also be used as an elution buffer for ET Terminator samples, though it may reduce the signal of your samples. When using formamide as an elution buffer, seal and shaking the plates for 30~60 seconds before loading them. Additionally, when formamide absorbs moisture from the air, it creates formic acid, which can break apart some of the incorporated dyes and cause dye blobs.

10. Place the reaction plate onto an magnetic plate to separate beads from solution. When separation is complete, transfer cleared samples into a clean plate.
    The recommended separation time is 3 minutes. The solution should be clear before transferring samples to the new plate. It is very important to remove the beads as completely as possible prior to loading. Leaving 5μL of liquid behind in the elution plate can help to prevent transfer of beads into the loading plate.