Manual

Version 5.1

Product name: Dye Sequencing Clean Up Kit

Cat #: BCB-100, BCB-200, BCB-250, BCB-300

Description

MCLAB's Dye Sequencing Clean Up Kit, based on carboxylated magnetic bead technology, delivers a higher yield after the dye terminator removal at a fraction of the cost.* With its high sequencing pass rates and longer Phred 20 read lengths (> 900 base pairs on average), the Big-Dye Sequencing Clean Up Kit is superior to alternative cleanup methods like ethanol precipitation, gel filtration or silica-based magnetic reagents adsorption.

Purifcation Method

1. Manually/centrifuge module with magnetic beads
2. Manually/pipette module with magnetic beads

Manually module (96-well format)

Items to be prepared before starting:

1. Centrifuge with microplus carrier
2. 96-well magnet plate
3. 96-well collection plate
4. Multichannel pipette (10µl-100µl)
5. 80% Ethanol
6. 70% Ethanol
7. Elution buffer (1X) /ddH_2O

By following the protocol (typically 10µl reaction system), you can increase or decrease in proportion to your needs

1. Remove the 96-well plate from the PCR machine and spin slightly.
2. Remove the cover from the reaction plate.
3. Shake the beads bottle to fully re-suspend the magnetic beads before usage
4. Prepare the binding system.
   a. Add 10µl beads to each sample (10µl).
   b. Add 40µl of 80% ethanol to each sample.
   c. Pipette the system 3-5 times, mix well.
5. Loading the binding system to a magnet plate and keep it for 1 minute.

6. Discard the supernatant as follows:

For Manually/centrifuge module

a. Invert the reaction plate with the magnet plate together onto a paper towel folded to the size of the plate.

b. Place the inverted three layers system into the centrifuge and spin up to 300 rpm, then remove from the centrifuge.

c. Discard the wet paper towel; be careful do not separate the reaction plate from the magnet plate.

For Manually/pipette module

a. Keep the reaction plate on the magnet plate.

b. Aspirate the cleared solution (supernatant) from the plate and discard it.

NOTE:

1. To avoid disturbing the beads, it is better to place the pipette tip at the bottom of the well when aspirating.
2. Remove as much supernatant as possible because it contains excess fluorescent dye and contaminants.

7. Perform a 70% ethanol wash.

a. Add 80µl of 70% ethanol to each well.

b. Pipette the system 3-5 times, mixing well.

c. Hold the washing system for 1 minute.

8. Discard the washing supernatant as follows:
Repeat the Step 6.

9. Perform DNA elution.

a. Add 40µl of elution buffer to each well.

b. Remove the reaction plate from the magnet plate.

c. Pipette the system 3-5 times, mixing it well.

d. Re-load the reaction plate to a magnet plate and hold for 1 minute.

10. Pipette 25µl of the eluting liquid to the collecting plate and spin slightly.

11. Loading a septa onto the collecting plate; now it is ready for capillary electrophoresis.

*Compared to the popular carboxylated bead based kits such as the Agencourt's CleanSEQ®.