MCLAB

Big Dye Sequencing Clean up kit

# User Manual

320 Harbor Way
South San Francisco, CA 94080
Phone: 1 (888) MCLAB-88
Fax: 1 (650) 871-8796
www.mclab.com

# Contents



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## *Manual protocol (96 well format)*

1. Resuspend Bigdye Cleaning Bead (BCB) by shaking.
2. Add 10 uL BCB into each sample.
3. Add 85% ethanol into each sample and thoroughly mix by pipetting. Add ethanol based on the equation below:
   85% ethanol volume (uL) = 2.077 x (10 + sample volume (uL))
4. Place sample plate onto the 96well magnetic plate and wait for 3 minutes or until the solution is clear.
5. While the plate is on the magnet, aspirate the solution (supernatant) from the sample wells and discard.
6. Add 100 uL of 85% ethanol into each well and wait 30 seconds.
   It is important to do this step while the plate is situated on the magnetic plate. There is no need to resuspend the beads.
7. Aspirate ethanol and discard.
   It is important to do this step while the plate is situated on the magnetic plate. Remember to fully remove the ethanol as it contains contaminants.
8. Repeat steps 6 and 7 for a total of two ethanol washes.
9. Air-dry sample in room temperature for 10 minutes. Do not over dry as it can degrade the fluorescent dye. Sample plate can be placed on or off the magnetic plate while drying.
10. Add 40 uL of elution buffer (0.1 mM EDTA pH 8.0 or DiH2O) and mix thoroughly by pipetting. Incubate at room temperature for 5 minutes. Sample plate must be off the plate for elution. Make sure beads are fully resuspended after mixing.
11. Place sample plate on magnetic plate and wait 3 minutes or until solution clears. While keeping sample plate on the magnet, transfer 35 uL of cleared solution onto a new plate.
    5 uL – 10 uL is left behind to prevent bead transfer as it can interfere with injection. If beads do transfer, place samples back onto original plate and re-transfer onto new plate.