

MICROMON SANGER SEQUENCING

CYCLE SEQUENCING REACTION CLEAN UP - TUBE FORMAT

SODIUM ACETATE-ETHANOL METHOD

*****All reagents must be at Room Temperature**

In a standard 1.5ml microfuge tube:

3 μ l 3M Sodium Acetate pH 5.2

62.5 μ l Ethanol (commercial 96% (v/v) Analytical grade stock)*

14.5 μ l RO grade water

OR

3 μ l 3M Sodium Acetate pH 5.2

77 μ l Ethanol (78% (v/v) solution)*

80 μ l Total

Note: 3M sodium acetate is prepared using sodium acetate trihydrate and lowering the pH to 5.2 with glacial acetic acid.

- Add the 20 μ l cycle sequencing reaction to the sodium acetate/ethanol solution in the 1.5ml tube and follow precipitation procedure. It might be necessary to use two 70% wash steps if the unincorporated dye terminators are persistent or a clean read nearer to the primer is needed.

- Starting with the 100 μ l cycle sequencing reaction solution from above:

- Vortex

- Stand for 15 minutes at room temperature

- Centrifuge at 13,000 rpm for 20–30 minutes (tube hinge outwards)

- Using a 1–200µl pipette tip, carefully place the tip in the supernatant on the non-hinged side of the tube, gradually removing the liquid right down to the bottom of the tube (do not leave any liquid behind at all)
- Add 200µl of 70% (v/v) Ethanol
- Vortex
- Centrifuge at 13,000 rpm for 3–5 minutes
- Again using a pipettor, carefully remove all the liquid as previously described
- With the lid open, stand the tube in a heat block at 70–90°C for 1 minute
- Allow to cool, close lid and store in the dark in a refrigerator

*** It is most important that a commercially prepared, non-denatured 95-96% Analytical Reagent Grade Ethanol stock is used for this clean up procedure. It is very important that the final level of Ethanol in the precipitation step is as close to 60% (v/v) as possible. We recommend Ajax product #1046 or similar. Absolute Ethanol should not be used because it is extremely hygroscopic and will absorb water from the atmosphere over time, resulting in unknown water content. The wash solution is prepared by diluting the Ethanol stock to 70% (v/v) with RO grade water.**

Suggested Ethanol products:

Chem-Supply Ethanol 95% EA042-2.5L-P (2.5 litre plastic bottle)

Agency: Pacific Laboratory Products

Ajax FineChem Ethanol 96% 1046-2.5L-PL (2.5 litre plastic bottle)

Agency: Thermo Fisher Scientific