

# MICROMON SANGER SEQUENCING

## CYCLE SEQUENCING REACTION CLEAN UP - PLATE FORMAT

### SODIUM ACETATE-ETHANOL METHOD

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

To each well of the plate that contains a 20 µl processed reaction add the following:

3 µl 3M Sodium Acetate pH 5.2

77 µl 78% Ethanol (made using commercial 96% (v/v) Analytical grade stock)\*

80 ul Total

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

Mix the contents of each well, either by use of a multichannel pipettor or by a vortex mixer with a plate adapter (sealing mat must be in position if vortexing).

- Remove any bubbles from the base of wells as necessary
- Seal the plate using the silicone sealing mat
- Stand for 15 minutes at room temperature
- Centrifuge at 1,500 g for 45 minutes to pellet the DNA (set the run temperature to 20 degrees)
- Immediately remove the mat and gently shake out the supernatant in the sink – dry the top of the plate with a tissue
- Invert the plate on the tissue in the centrifuge and spin at 185 g for 15 seconds
- Add 100 µl of 70% (v/v) Ethanol to each well
- Vortex plate for 15 seconds
- Centrifuge at 1,500 g for 10 minutes
- Immediately, gently shake out the supernatant in the sink, then dry the top of the plate with a tissue

- Invert the plate on a tissue in the centrifuge and spin at 185 g for 15 seconds
- Stand the plate in a heat block at 70–90°C for 60 seconds
- Allow to cool

**\* 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 an 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**