

# **Medium Preparation**

## **R & F® non-O157 STEC Chromogenic Medium**

### **Plating Medium Powder**

Add 57.0 grams of powder to 1 liter of distilled or deionized water. Stir to dissolve any clumps and powder from the surface of the container. Adjust pH to 6.7-6.9 at 25°C using either 1 N NaOH or HCL. Boil to dissolve completely. Avoid overheating. DO NOT AUTOCLAVE. Cool to 50-55°C in a waterbath.

Prepare R & F® non-O157 STEC supplements for the chromogenic plating medium following instructions. After the solutions are filter sterilized, aseptically add 1.0 ml from M-17A (sodium cefsulodin) and 0.5 ml from M-17B (sodium novobiocin) to the cooled plating medium. Gently swirl the medium to disperse the solutions in the medium. Return remaining solutions of vials M-17A and M-17B to the cooler at 2-8°C. Pour the completed medium into Petri plates and allow the surface to dry by keeping the plates at room temperature 1 to 2 days in the dark. Store the plates in Petri plate vented sleeves in the dark at 2-8°C for up to 60 days. Final pH 6.70 to 6.90 at 25°C

### **Supplement Powders**

The contents contain twelve (12) vials including 6 vials labeled M-17A (3 vials labeled sodium cefsulodin and 3 sterile vials for cefsulodin solution), 6 vials labeled M-17B, (3 vials labeled sodium novobiocin and 3 sterile vials for novobiocin solution). Pipette 5 ml of sterile deionized or distilled water into one of the M-17A and M-17B vials labeled sodium cefsulodin and sodium novobiocin, respectively. Secure the cover and shake the contents of each vial until the powders are completely dissolved. Do not use heat. Separately filter sterilize the solutions of each vial using a 0.45 or 0.20 µm pore size filter. Aseptically pour the sterile cefsulodin and novobiocin solutions into respective sterile empty M-17A and M-17B vials. CAUTION: BE SURE NOT TO MIX SOLUTIONS OR POUR INTO THE WRONG VIALS. Date the vials with the date sterilized and 3 months from sterilization as the expiration date. Store vials M-17A and M-17B at 2-8°C in the dark until use. Follow the directions on the R & F® non-O157 STEC Chromogenic Plating Medium bottle label to make 1 liter of medium. For less than 1 liter adjust the solution amounts to the medium accordingly.