Medium Preparation

**R & F® Escherichia coli O157:H7 Enrichment Broth**

**Enrichment Broth Powder**
Add 45.6 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 7.00-7.10 at 25°C using either 1 N NaOH or HCl. Sterilize at 121-124°C for 15 minutes. After cooled, secure the cap. The enrichment broth without supplements can be stored at room temperature in the light for up to 3 months using a secure cap.

Prepare R & F® *Escherichia coli* O157:H7 supplements for the enrichment broth following instructions. After the solutions are filter sterilized, aseptically add 0.4 ml from M-2A (3-Hydroxy-2-Methyl-4-Pyrone; Maltol), 1.0 ml from M-2B (sodium cefsulodin) and 0.8 ml from M-2C (vancomycin hydrochloride) to 1 liter of cooled enrichment broth. Gently swirl the broth to disperse the solutions in the medium. Return remaining solutions of vials M-2A, M-2B and M-2C to the cooler at 2-8°C. Any unused portion of the complete enrichment broth can be stored at 2-8°C in the dark for up to 45 days. Final pH 6.85 to 7.15 at 25°C.

**Supplement Powders**
The contents contain twelve (12) vials including 4 vials labeled M-2A (2 vials labeled 3-Hydroxy-2-Methyl-4-Pyrone [Maltol] and 2 sterile vials for 3-Hydroxy-2-Methyl-4-Pyrone solution), 4 vials labeled M-2B (2 vials labeled sodium cefsulodin and 2 sterile vials for cefsulodin solution), and 4 vials labeled M-2C (2 vials labeled vancomycin hydrochloride and 2 sterile vials for vancomycin solution). Pipette 6 ml of sterile deionized or distilled water into one of the M-2A, M-2B and M-2C vials labeled 3-Hydroxy-2-Methyl-4-Pyrone (Maltol), sodium cefsulodin and vancomycin hydrochloride, 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 3-Hydroxy-2-Methyl-4-Pyrone, cefsulodin and vancomycin solutions into respective sterile empty M-2A, M-2B and M-2C 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-2A, M-2B and M-2C at 2-8°C. Follow the directions on the R & F® *Escherichia coli* O157:H7 Enrichment Broth bottle label to make 1 liter of broth.