

Medium Preparation

R & F® *Bacillus cereus* Group Enrichment Broth

Enrichment Broth Powder

Add 35.6 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 7.10-7.40 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 the supplement can be stored at room temperature in the dark for up to 3 months using a secure cap.

Prepare R & F® *Bacillus cereus* Group supplement for the enrichment broth following instructions. After the solution is filter sterilized, aseptically add 2.0 ml from M-21A Solution Vial (Polymyxin B) and 2.0 ml from M-21B Solution Vial (ceftazidime) to 1 liter of cooled enrichment broth. Gently swirl the broth to disperse the solution in the medium. Return remaining M-21A and M-21B solution vials 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 6.80 to 7.20 at 25°C

Supplement Powders

The contents contain twelve (12) labeled vials, including 6 vials labeled M-21A (3 vials labeled polymyxin B sulfate and 3 sterile vials for M-21A polymyxin B solution) and 6 vials labeled M-21B (3 vials labeled ceftazidime pentahydrate and 3 sterile vials for ceftazidime solution). Pipette 12 ml of sterile deionized or distilled water into one of the M-21A vials and one of the M21B vials until the powders are completely dissolved. Do not use heat. Separately filter sterilize the solutions of each vial using 0.45 or 0.20 µm pore size filter. Aseptically pour the sterile polymyxin B sulfate and ceftazidime solutions into respective sterile empty M-21A and M-21B solution 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 containing the solutions at 2-8°C until use. Follow the directions on the R & F® *Bacillus cereus* Group Enrichment Broth bottle label to make 1 liter of broth.