**Medium Preparation**

**R & F® Enterobacter sakazakii (Cronobacter) Chromogenic Medium**

**Plating Medium Powder**
Add 62 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 6.90-7.00 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® Enterobacter sakazakii supplements for the chromogenic plating medium following instructions. After the solutions are filter sterilized, aseptically add 0.6 ml from M-7A (sodium cefsulodin) and 0.8 ml from M-7B (vancomycin hydrochloride) to the cooled plating medium. Gently swirl the medium to disperse the solutions in the medium. Return remaining solutions of vials M-7A and M-7B 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 45 days. Final pH 6.80 to 7.00 at 25°C

**Supplement Powders**
The contents contain eight (8) vials including 4 vials labeled M-7A (2 vials labeled sodium cefsulodin and 2 sterile vials for cefsulodin solution) and 4 vials labeled M-7B (2 vials labeled vancomycin hydrochloride and 2 sterile vials for vancomycin solution). Pipette 3 ml of sterile deionized or distilled water into one of the M-7A vials and one of the M-7B vials labeled 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 cefsulodin and vancomycin solutions into respective sterile empty M-7A and M-7B 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® Enterobacter sakazakii Chromogenic Plating Medium bottle label to make 1 liter of medium. For less than 1 liter adjust the solution amounts to the medium accordingly.

**R & F® Enterobacter sakazakii Screening Medium**

**Plating Medium Powder**
Add 6.8 grams of powder to 235 ml of distilled or deionized water. Prepare two separate containers. Stir to dissolve any clumps and powder from the surface of the containers. Adjust pH to 6.70-6.90 at 25°C using either 1 N NaOH or HCL. Sterilize at 121-124°C for 15 minutes. Allow the media in the containers to cool to 50-55°C in a waterbath.

Prepare R & F® Enterobacter sakazakii supplements for the screening plating medium following instructions. After the solutions are filter sterilized, aseptically add 15 ml from M-7C (melibiose) to one cooled medium and 15 ml from M-7D (sucrose) to the other cooled medium yielding a final 0.5% solution of each carbohydrate. Swirl the medium to disperse the solution in the medium. To one section of a biplate Petri plate pour the medium containing melibiose and to the other section pour the medium containing the sucrose. Label the melibiose side "M" and the sucrose side "S". Allow the surface to dry by keeping the biplate at room temperature 1 to 2 days. Store the plates in Petri plate sleeves in the dark at 2-8°C for up to 45 days.
Medium is a rapid preliminary confirmation of presumptive colonies from R & F® Enterobacter sakasakii Chromogenic Plating Medium. A biplate can accommodate up to 3 presumptive colonies. Divide the biplate into three compartments. Each compartment uses one colony. Inoculate each compartment with a straight streak. Incubate the biplate at 35°C for 4 to 6 hrs. Examine for yellowing around bacterial growth indicating acid production. If both sections of the biplate per colony are yellow, confirm conventionally. If either section of the biplate per colony is not yellow, re-incubate overnight. Per colony, if one or both sections of the biplate are not yellow no further confirmation is required. Enterobacter sakasakii produces acid from both melibiose and sucrose, therefore both section of the biplate need to be yellow around the bacterial growth to pursue final confirmation. Final pH 6.70 to 6.90 at 25°C

Supplement Powders
The contents contain eight (8) vials including 4 vials labeled M-7C (2 vials labeled melibiose and 2 sterile vials for melibiose solution) and 4 vials labeled M-7D (2 vials labeled sucrose and 2 sterile vials for sucrose solution). Pipette 30 ml of sterile deionized or distilled water into one of the M-7C vials and one of the M-7D vials labeled melibiose and sucrose, 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 melibiose and sucrose solutions into respective sterile empty M-7C and M-7D 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® Enterobacter sakazakii Screening Plating Medium for Preliminary Confirmation bottle label to make 250 ml of medium for each carbohydrate.