Medium Preparation

R & F® Listeria sp./Listeria monocytogenes Chromogenic Medium

Plating Medium Powder
Add 1.5 grams of powder from vial M-11D contained in the R & F® Listeria sp./Listeria monocytogenes supplement for chromogenic plating medium to 485 ml of distilled or deionized water. Gently, but thoroughly, swirl to disperse the powder forming a chalky white solution. Return the remaining powder in vial M-11D to the supplement box and freeze. Add 31.0 grams of powder from the chromogenic plating medium powder bottle to the chalky white solution. Warm slightly and stir to dissolve any clumps and powder from the surface of the container. Sterilize at 121-124°C for 12 minutes. IMPORTANT: DO NOT AUTOCLAVE BEYOND 12 MINUTES. Allow to cool in a waterbath at 50°C.

Prepare R & F® Listeria sp./Listeria monocytogenes supplement for the chromogenic plating medium following instructions. After the solution in vial M-11C is filter sterilized, aseptically add the total volume (15ml) to the cooled chromogenic plating medium. Gently swirl the medium to dispense the solution in the medium and also making sure the white precipitate is thoroughly dispersed in the medium. Pour the completed medium into Petri plates. Gently swirl intermittently during pouring. 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 6.90 to 7.20 at 25°C.

Supplement Powders
The contents contain thirteen (13) labeled vials, including 4 vials each labeled M-11A, M-11B and M-11C and one vial M-11D. One vial of each M-11A, M-11B and M-11C is necessary for making the complete supplement for 500 ml of the R & F® Listeria sp./Listeria monocytogenes Chromogenic Plating Medium. Pour the powder in vial M-11A into a clean beaker containing 15 ml of distilled or deionized water. Add a stirring bar to the beaker and stir the contents of the beaker until the powder is completely dissolved. The solution should appear clear-light yellow. Make sure that no powder remains on the side of the beaker or floating on the top of the solution. Do not use heat.

Pour the clear-light solution into vial M-11B making sure no solution remains in the beaker. Excess foam can be pipetted from the beaker. Secure the cover and shake the contents of vial M-11B until the powder is completely dissolved. The powder should dissolve quite easily. Allow vial M-11B to remain still for a few minutes. No powder should be observed on bottom of vial M-11B.

Pour the solution from vial M-11B into vial M-11C. Secure the cover and shake the contents of vial M-11C until the powder in the vial M-11C is dissolved. The powder should dissolve quite easily. Allow vial M-11C to remain still for a few minutes. No powder should be observed on the bottom of vial M-11C.

Filter sterilize the contents in vial M-11C into sterile container using a 0.45 or 0.20 µm filter. Aseptically pour the sterile supplement into a cooled (50°C) 485 ml of R & F® Listeria sp./Listeria monocytogenes Chromogenic Plating Medium. If making less than 1/2 liter of Listeria sp./Listeria monocytogenes Chromogenic Plating Medium, the remaining sterile M-11C solution can be stored at 2-8°C for up to 30 days in the dark.

R & F® Listeria monocytogenes Confirmatory Medium
**Plating Medium Powder**

Add 10.1 grams of powder to 250 ml of distilled or deionized water. Warm slightly and stir to dissolve any clumps and powder from the surface of the container. Sterilize at 121-124°C for 15 minutes. Allow to cool in a waterbath at 50°C. Gently swirl, pour the medium into one section of a Petri biplate. Prepare Purple Broth Base per manufacturer's instructions. Add 2.00% agar to the base, autoclave, and allow cooling at 50°C. Add filter sterilized rhamnose at a final concentration of 1.0%. Pour the medium into the other section of the Petri biplate. Allow the surface to dry by keeping the biplates at room temperature in the dark overnight. Store the plates in Petri plate sleeves in the dark at 2-8°C for up to 60 days.

The purpose of both plating medium is to differentiate *Listeria monocytogenes* from *Listeria ivanovii* which are the only *Listeria* sp. producing turquoise colonies and blue-violet/blue-green colonies on the R & F® *Listeria monocytogenes* and R & F® *Listeria sp./Listeria monocytogenes* Chromogenic Plating Media, respectively. Ninety-eight percent of the *Listeria monocytogenes* are positive for the R & F® *Listeria monocytogenes* Confirmatory Medium (alpha-mannosidase indicated by a fluorogenic reaction and acid from rhamnose), whereas *Listeria ivanovii* is negative. Therefore either reaction can be positive for *Listeria monocytogenes* confirmation. Final pH for both media 7.00 to 7.20 at 25°C