



USE PROTOCOL FOR THE R & F[®] *BACILLUS CEREUS* GROUP ENRICHMENT BROTH AND CHROMOGENIC PLATING MEDIUM

- 1 Prepare the R & F[®] *Bacillus cereus* Group Enrichment Broth according to the instructions provided on the packaging label. After the broth has been prepared, it can be stored at room temperature in the light for up to 3 months. After antibiotics have been aseptically added to the broth, any unused portions can be stored at 2-8°C in the dark for up to 45 days. Prepare the R & F[®] *Bacillus cereus* Group Chromogenic Plating Medium according to the instructions provided on the packaging labels. After the plates have been poured, they should be stored in the dark for 48 hours at room temperature to dry the surface of the agar. After surface drying, the plates can be placed in Petri plate sleeves (cutting holes in the sleeves to allow condensation to escape) and store inverted in the dark at 2-8°C for up to 60 days.
- 2 The food product should be diluted 1:10 using the completed R & F[®] *Bacillus cereus* Group Enrichment Broth. The food homogenate should be thoroughly mixed manually or mechanically for at least 2 minutes depending on the food matrix being analyzed. For an environmental sponge sample, add 100 ml of R & F[®] *Bacillus cereus* Group Enrichment Broth and massage vigorously for 1 minute.
- 3 Incubate the broth for 24-28 hours at 30°C.
- 4 After incubation, use a sterile inoculating loop (10 µl) to streak the enriched sample onto the surface of the prepared R & F[®] *Bacillus cereus* Group Chromogenic Plating Medium.
- 5 Incubate the plate for 24-28 hours at 35°C.
- 6 *Bacillus cereus* Group colonies appear medium to dark turquoise; flat or convex; dull to glassy; irregular to circular colonies with or without a cream ring. Some strains of *B. pseudomycoides* or *B. mycoides* may appear as rhizoid-shaped colonies with a dark turquoise center.
- 7 Use standard methods for colony confirmation.