



## USE PROTOCOL FOR THE R & F® *ENTEROBACTER SAKAZAKII* CHROMOGENIC PLATING AND SCREENING MEDIA

- 1 Prepare the R & F® *Enterobacter sakazakii* Chromogenic Plating Medium (ESPM) according to the instructions provided on the packaging label. 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 a hole in the sleeves to allow condensation to escape) and stored at 2-8°C for up to 45 days.
- 2 The inoculation of the plates should use the streak plate technique by placing the inoculum on the surface of the plate and streaking it using a sterile inoculating loop.
- 3 Incubate the plates inverted for 24 hours at 35-37°C. For greater selectivity, incubate plates inverted at 41-42°C for 24 hours.
- 4 *Enterobacter sakazakii* colonies appear blue-black to blue-gray domed 1.0 to 2.0 mm in diameter with and without a clear halo. Other colony types appearing on this plating medium that are **not** presumptive *Enterobacter sakazakii* strains are: green with and without a green precipitate, yellow with or without a yellow precipitate, white, or clear.
- 5 With an inoculating loop, pick presumptive colonies from ESPM and inoculate both sides of a R & F® *Enterobacter sakazakii* screening biplating medium and streak a tryptic soy agar plate. Each biplate can accommodate up to 3 presumptive colonies.
- 6 Incubate the biplate at 35°C for 4 to 6 hours. Incubate the tryptic soy agar plate at room temperature under the light overnight.
- 7 After 4 to 6 hours, examine the biplates for yellowing around bacterial growth indicating acid production. If both sections of the biplate per colony show yellowing, confirm using conventional methods from the tryptic soy agar plate. If either section of the biplate per colony is not yellow, re-incubate overnight.
- 8 Per colony, after 24 hours if one or both sections of the biplate are not yellow, no further confirmation is required. If both are yellow per colony, use the growth on the tryptic soy agar plate for conventional confirmation.