



USE PROTOCOL FOR THE R & F® *ENTEROBACTER SAKAZAKII* ENRICHMENT BROTH, PLATING AND SCREENING MEDIA

- 1 Prepare the R & F® *Enterobacter sakazakii* Enrichment Broth (ESEB) 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 the 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® *Enterobacter sakazakii* Chromogenic Plating Medium (ESPM) and the R & F® *Enterobacter sakazakii* Screening Medium (ESSM) 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 stored at 2-8°C for up to 45 days.
- 2 The food product should be diluted 1:10 using the completed ESEB. This food homogenate should be thoroughly mixed manually or mechanically depending on the food product. For a sponge, add 100 ml of ESEB and massage for 1 minute.
- 3 Incubate the enrichment broth at 35-37°C.
- 4 After 24 and 48 hours incubation, use a loopfull of the broth to streak the surface of the ESPM plate.
- 5 Incubate the plates inverted for 24 hours at 35-37°C. For greater selectivity, incubate the plates inverted at 41-42°C for 24 hours.
- 6 *Enterobacter sakazakii* colonies appear blue-black to blue-grey domed 1.0 to 2.0 mm in diameter with or without a clear ring. Other colony types appearing on this plating medium that are **not** presumptive *Enterobacter sakazakii* strains are: green with or without a green precipitate, yellow with or without a yellow precipitate, white or clear.
- 7 With an inoculating loop, pick presumptive colonies from ESPM and inoculate both sides of an ESSM bi-plate medium and streak a tryptic soy agar (TSA) plate. Each bi-plate can accommodate up to 3 presumptive colonies.
- 8 Incubate this bi-plate at 35°C for 4 to 6 hours. Incubate the TSA plate at room temperature in the light overnight.
- 9 After 4 to 6 hours, examine the bi-plates for yellowing around bacterial growth indicating acid productions. If both sections of the bi-plate per colony show yellowing, confirm using conventional methods from the TSA plate. If either section of the bi-plate per colony is not yellow, re-incubate overnight.
- 10 Per colony, after overnight incubation if one or both sections of the bi-plate are not yellow, no further confirmation is required. If both sides are yellow per colony, use the growth on the TSA for conventional confirmation.