

ENTEROBACTER SAKAZAKII ISOLATION CHROMOGENIC AGAR (ESIA) ISO 22964

CAT N°: 1446 For the isolation of presumptive *Enterobacter sakazakii* in infant milk products

FORMULA IN g/l

Pancreatic Digest of Casein	7.00	α -D-glucopyranoside	0.15
Sodium Chloride	5.00	Crystal Violet	0.002
Yeast Extract	3.00	Bacteriological Agar	15.00
Sodium Desoxycholate	0.60		

Final pH 7.0 \pm 0.2 at 25°C

PREPARATION

Suspend 30.7 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50°C, homogenize gently and dispense into Petri dishes in amounts of 15 ml. The prepared medium should be stored at 8-15°C. The color is purplish blue.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

USES

ENTEROBACTER SAKAZAKII ISOLATION CHROMOGENIC AGAR (ESIA) is a selective medium for the detection of *Enterobacter sakazakii* in milk powder and powered infant formula. The ISO normative 22964 recommends this medium for the isolation of *Enterobacter sakazakii*.

Casein peptone provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Sodium desoxycholate and Crystal violet inhibit gram-positive bacteria. 5-Bromo-4-chloro-3-indolyl α -D-glucopyranoside is the chromogenic substrate.

Enterobacter sakazakii is currently considered an emerging pathogen responsible for, un-weaned babies, risking severe meningitis and necrotic enterocolitis that can be the cause of mortality rate between 40-80%.

The pathogenicity of *Enterobacter sakazakii* for un-weaned babies' makes it necessary to review the manufacturing process of the milk-based products specialized for babies, guaranteeing the absence of the bacteria in the final product. Additional prevention measures at a hospital include the sanitary hygiene of the prepared food; reducing the time between the preparation and its administration, to impede the multiplication of microorganisms.

After incubation of the inoculated Lauryl Sulphate Tryptose Broth Modified (mLST) (Cat. 1445), streak a loopful (ca. 10 μ l) onto the surface of the *Enterobacter sakazakii* isolation chromogenic agar plate. Incubate the plate at 44 °C \pm 1 °C for 24 h \pm 2 h. After incubation, examine the chromogenic plate for the presence of typical colonies of presumptive *Enterobacter sakazakii*. Typical colonies are small to medium sized green to blue-green colonies. Non-typical colonies are often slightly transparent and violet coloured. The typical colony colors of green – blue greenish should be confirmed in TSA Agar (Cat. 1068) in which they present yellow color. A biochemical confirmation is needed from the yellow pigmented colonies.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of $44^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and observed after 24 ± 2 hours

Microorganisms	Growth	Colony Color
<i>Escherichia coli</i> ATCC 25922	Good	Transparent – red/violet
<i>Escherichia coli</i> ATCC 8739	Good	Transparent – red/violet
<i>Enterobacter sakazakii</i> ATCC 29544	Good	Green-blue - greenish
<i>Staphylococcus aureus</i> ATCC 25923	Inhibited	
<i>Enterococcus faecalis</i> ATCC 29212	Inhibited	

BIBLIOGRAPHY

ISO normative 22964:2006 Milk and milk products detection of *Enterobacter sakazakii*

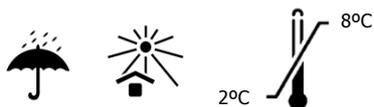
GUILLAUME-Gentil, O., Sonnard, V. Kandahai, M.C., Mauragg, J.D. and Jootsen, H. A simple and Rapad Cultural Method for Detection of Enterobacter Sakazakii in environmental samples. Journal of Food.

Protection, 68 (1), 2005, pp. 64-69.



STORAGE

Once opened keep powdered medium closed to avoid hydration.



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