chromocult

High safety at a low price.

ChromoCult® Enterobacter sakazakii Agar
for cost-effective and safe detection
of E. sakazakii in food.

MERCK

chromocult

Colour makes the difference.

How a change of colour can save lives.

No matter how accurate infant milk formula is produced, it will not be sterile and may occasionally contain pathogens. In the past, *Enterobacter sakazakii* found in powdered infant formula was associated with outbreaks of severe diseases, such as meningitis, in neonates and babies. Meningitis is extremely hazardous for infants because of its high mortality rate: Up to 50% of the infants, who contract the disease, die. And the survivors often suffer from critical and long lasting complications including neurological disorders.

The risk of contamination can easily be eliminated for a very small price. Merck offers the ChromoCult® Enterobacter sakazakii Agar to increase the security in detecting this microorganism in milk powder and infant formula. Thanks to its chromogenic detection system, based on the alpha-D-Glucosidase – an enzyme specific for *E. sakazakii* – only the colonies of *E. sakazakii* appear turquoise while other bacteria grow colourless. And because the combination of inhibitors and high incubation temperature (44°C) repress efficiently the growth of accompanying bacteria, ChromoCult® Enterobacter sakazakii Agar allows a fast and reliable detection within only 24 hours.

Your benefits

- **Best value for money**
  Reliable, fast and safe detection of *Enterobacter sakazakii* with a chromogenic medium for a low price per plate.

- **Highest security**
  The unique chromogenic technique is the only way to detect all *Enterobacter sakazakii* safely and clearly.

- **Most simplified analyses**
  Easy interpretation within 24 hours.
  Only *Enterobacter sakazakii* leads to turquoise coloured colonies while other bacteria are repressed or grow colourless.

ChromoCult® Enterobacter sakazakii Agar

Ord. No. 1.00873.0500 (500 g)

Selective medium for the detection of *Enterobacter sakazakii* in milk powder and powdered infant formula.

Mode of Action

The base in ChromoCult® Enterobacter sakazakii Agar allows good growth and strong color formation of *E.sakazakii* colonies.
The addition of inhibitors and the incubation temperature of 44 °C largely reduce the growth of the majority of Gram-positive and Gram-negative accompanying flora.
By adding 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside a differentiation of α-D-glucosidase-positive and -negative bacteria is possible.
E.sakazakii is α-D-glucosidase positive and grows as blue-green colonies on this medium.

Typical Composition (g/Liter)

Peptone 6.0; sodium chloride 5.0; bile salt mixture 1.5; 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside 0.1; agar agar 12.0.

Preparation

Suspend 24.6 g in 1 litre of demin. water by heating in a boiling water bath or in flowing steam until the medium is completely dissolved. Autoclave at 121°C for 15 min. Cool to 45 - 50°C in a water bath, mix gently and pour about 15 ml in sterile Petri dishes. pH: 7.0 ±0.2 at 25°C. The prepared medium is clear and slightly yellow. The prepared plates can be stored for up to 2 weeks at +2°C to +8°C (protect from light and dehydration).

Experimental Procedure and Evaluation

The agar plates must be dry. In the case of visible water dry them before use (e.g. 20 min. at 55°C).
Inoculate the medium with a loopful of enrichment broth, streaking for isolation. Incubation: 24 ±2 hours at 44 ±1°C.
Note: Incubation temperature has a strong influence on sensitivity and selectivity of this method. Temperatures higher than 45°C will inhibit the growth of *E.sakazakii*. Temperatures below 43°C will reduce inhibition of accompanying bacteria. Pre-heat the incubator to 44°C. Do not overload the incubator. Do not stack dishes more than three high.
Results: *E.sakazakii*: blue green colonies.
Accompanying bacteria: colourless colonies.

Quality Control

Test strains	Recovery rate	Colour of colony
Enterobacter sakazakii ATCC 29544	> 70 %	blue-green
Enterobacter sakazakii ATCC 29004	> 70 %	blue-green
Enterobacter cloacae ATCC 29941	not limited	white
Proteus mirabilis ATCC 29906	not limited	white
Escherichia coli ATCC 11775	not limited	white
Enterococcus faecalis ATCC 11700	< 0.001 %	-
Staphylococcus saprophyticus ATCC 15305	< 0.001 %	-

Ordering Information

Product	Ord. No.	Pack size
ChromoCult® Enterobacter sakazakii Agar	1.00873.0100	100 g
ChromoCult® Enterobacter sakazakii Agar	1.00873.0500	500 g
Buffered Peptone Water	1.07228.0500	500 g
Lauryl Sulfate Broth (LST)	1.10266.0500	500 g
Sodium Chloride	1.06404.0500	500 g

Literature

• *ASM Meeting 2005, Atlanta, USA*
Comparison of three chromogenic media for detection of Enterobacter sakazakii; a preliminary study. M. Manafi and Kerstin Lang, Hygiene Institute, Medical University of Vienna, 10950 Vienna/Austria, 2005
• *Anti-bacteria & Anti-fungi Association academic meeting, Japan 2006*
Evaluation of simple and rapid detection of E. sakazakii by using chromogenic substrate media. Fumi Suzuki, Ken Noguchi, Rolf Ossmer, Merck Japan, 2006

chromocult

Colour makes the difference.

ISO 22964 Draft Protocol

Preparation

Pre-enrichment of
10 g sample in 90 ml BPW
18 h ± 2 h at 37°C
recommended by ISO 22964

Selective-enrichment in mLST-Vancomycin medium
0.1 ml from cultured BPW into 10 ml mLST-Vancomycin medium
24 ± 2 h at 45 ± 0.5°C
recommended by ISO 22964

Detection

Isolation on ChromoCult® Enterobacter sakazakii Agar
Streak a loop full cultured mLST-Vancomycin medium
on a plate of chromogenic agar
24 h at 44 ± 1°C

Counting and Confirmation

Turquoise colonies
Enterobacter sakazakii
on ChromoCult® Enterobacter sakazakii Agar

No further confirmation step is needed!

Colourless colonies
No *Enterobacter sakazakii*
on ChromoCult® Enterobacter sakazakii Agar

No further confirmation step is needed!

GRANULATED CULTURE MEDIA FOR YOUR HEALTH AND SAFETY

W 286152 03/07, 1st issue

Merck KGaA
64271 Darmstadt, Germany
Fax: +49 (0)61 51/72-60 80
E-mail: mibio@merck.de
microbiology.merck.de