* HiCrome™ Enterobacter sakazakii HiVeg™ Agar (HiCrome™ Cronobacter sakazakii HiVeg™ Agar)

MV1577

(Enterobacter sakazakii* now referred as *Cronobacter sakazakii*).**

Intended Use:

Recommended for the isolation and identification of *Enterobacter sakazakii* now referred as *Cronobacter sakazakii* from food and dairy products.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	15.000
Soya peptone	5.000
Sodium chloride	5.000
Synthetic detergent No. III	0.500
Sodium thiosulphate	1.000
Chromogenic mixture	10.170
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.67 grams in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Enterobacter species are widely distributed in nature, occurring in fresh water, soil, sewage, plants, vegetables, animal and human faeces. **Cronobacter sakazakii* has been closely associated with neonatal meningitis and sepsis (1). The chromogenic substrate in the media is cleaved specifically (2) by the glucosidase enzyme possessed by *Enterobacter* species resulting in formation of blue-green colonies. Other organisms, which do not cleave this substrate, produce yellow coloured colonies. Incorporation of the chromogenic mixture in the media renders an intense blue colour to **Cronobacter sakazakii* colonies whereas light blue green colour to the other *Enterobacter* species. HiCrome™ Cronobacter sakazakii HiVeg™ Agar is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones.

HiVeg™ hydrolysate and soya peptone provide nitrogenous and carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Synthetic detergent No. III inhibits the accompanying gram-positive flora.

Key: *: Formerly known as *Enterobacter sakazakii*

Type of specimen

Food and Dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4,5).
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Slight variation in colour may be observed depending on enzyme production by organism and substrate utilization from the medium.
2. Further biochemical tests must be carried out for confirmation.
3. Some species may show poor growth due to nutritional variations.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple coloured, clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 5.16% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=50%	yellow
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	good-luxuriant	>=50%	Green
Enterococcus faecalis ATCC 29212 (00087*)	>=10^4	inhibited	0%	
# *Klebsiella aerogenes* ATCC 13048(00175*)	50-100	good-luxuriant	>=50%	bluish green
##*Cronobacter sakazakii* ATCC 12868	50-100	good-luxuriant	>=50%	blue
Staphylococcus aureus subsp.*aureus* ATCC 25923 (00034*)	>=10^4	inhibited		

Key : *Corresponding WDCM numbers. # - Formerly known as *Enterobacter aerogenes* ##-Formerly known as *Enterobacter sakazakii*

Storage and Shelf Life

Store between 15-25°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

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3. Lipps WC, Braun-Howland EB, Baxter TE,eds. Standard methods for the Examination of Water and Wastewater, 24th ed. Washington DC:APHA Press; 2023.
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5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
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7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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