

HiCrome™ *Enterobacter sakazakii* Agar / Modified

Recommended for the isolation and identification of *Enterobacter sakazakii* from food, milk and dairy products (*Enterobacter sakazakii* now referred as *Cronobacter sakazakii*)

M1577/
M1641

Composition **		
	M1577	M1641
	Grams/Litre	Grams/Litre
Ingredients		
Tryptone	15.00	7.00
Soya peptone	5.00	-
Yeast extract	-	3.00
Sodium chloride	5.00	5.00
Sodium deoxycholate	0.50	0.60
Sodium thiosulphate	1.00	-
Chromogenic mixture	10.17	-
Chromogenic substrate	-	0.15
Crystal violet	-	0.002
Agar	15.00	15.00
Final pH (at 25°C)	7.3 ± 0.2	7.0 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.67 grams of M1577 or 30.75 grams of M1641 in 1000 ml 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 (3). The chromogenic substrate in HiCrome™ *Enterobacter sakazakii* Agar (M1577) 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 *C.sakazakii* colonies and light blue green colour to other *Enterobacter* species. HiCrome™ *Enterobacter sakazakii* Agar, Modified is recommended by ISO Committee for the isolation and identification of *C.sakazakii* (1). The chromogenic substrate is cleaved specifically (2) by *C.sakazakii* resulting in the formation of blue green colonies. Other organisms, which do not cleave this substrate, produce colorless to slightly violet coloured colonies.

Tryptone, soya peptone and yeast extract provide the essential growth nutrients along with nitrogenous and carbonaceous compounds, long chain amino acids and vitamins. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Sodium deoxycholate and crystal violet (in M1641) inhibits the accompanying gram-positive flora.

*: Formerly known as *Enterobacter sakazakii*

HiCromeVeg™ Freedom from BSE / TSE worries
Single Streak Rapid Differentiation Series

HiCrome™ Ent. sakazakii Agar (M1577) is also available as HiCrome™ Ent. sakazakii HiVeg™ Agar (MV1577) wherein all the animal origin nutrients have been replaced by vegetable based nutrients.

Type of specimen

Food, milk & dairy products

Specimen Collection and Handling

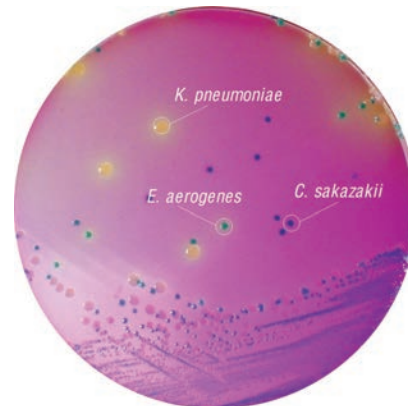
For food and dairy samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (4, 5, 6). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

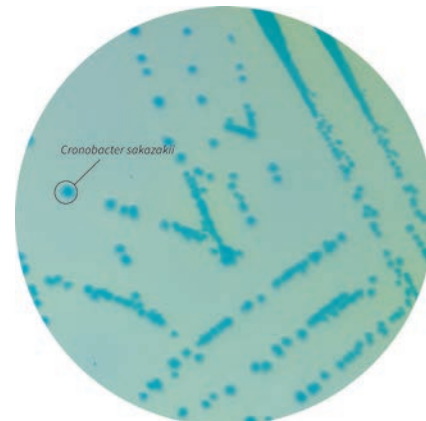
Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety 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. Some species may show poor growth due to nutritional variations.
3. Further biochemical tests must be carried out for confirmation.



M1577 HiCrome™ *Enterobacter sakazakii* Agar,



M1641 HiCrome™ *Enterobacter sakazakii* Agar, Modified

For identification of *Cronobacter (Enterobacter) sakazakii*

HiCrome™ *Enterobacter sakazakii* Agar / Modified

Recommended for the isolation and identification of *Enterobacter sakazakii* from food, milk and dairy products (*Enterobacter sakazakii* now referred as *Cronobacter sakazakii*)

M1577/
M1641

Performance and Evaluation

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

Quality Control

- Appearance of Powder** : Light yellow to pink coloured, homogeneous, free flowing powder.
- Gelling** : Firm, comparable with 1.5% Agar gel.
- Colour and Clarity of prepared medium** : Purple coloured (M1577) or light purple coloured (M1641), clear to slightly opalescent gel forms in Petri plates.
- Reaction** : Reaction of 5.16% w/v aqueous solution of M1577 at 25°C. pH : 7.3 ± 0.2 .
Reaction of 3.07% w/v aqueous solution of M1641 at 25°C. pH : 7.0 ± 0.2.
- Cultural Response** : M1577 Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> (25922) (00013*)	50-100	good-luxuriant	≥50%	yellow
▲ <i>Klebsiella aerogenes</i> (13048) (00175*)	50-100	good-luxuriant	≥50%	green
# <i>Cronobacter sakazakii</i> (12868)	50-100	good-luxuriant	≥50%	blue
<i>Klebsiella pneumoniae</i> (13883) (00097*)	50-100	good-luxuriant	≥50%	green (mucoid)
<i>Staphylococcus aureus</i> sub-sp <i>aureus</i> (25923) (00034*)	≥10 ³	inhibited	0%	—
<i>Enterococcus faecalis</i> (29212) (00087*)	≥10 ³	inhibited	0%	—

(M1641) : Cultural characteristics observed after an incubation at 44±1°C for 18-24 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> (25922) (00013*)	50-100	good-luxuriant	≥50%	colourless with blue centre

* <i>Cronobacter sakazakii</i> (12868)	50-100	good-luxuriant	≥50%	blue - green
<i>Staphylococcus aureus</i> sub-sp <i>aureus</i> (25923) (00034*)	≥10 ³	inhibited	0%	—
<i>Enterococcus faecalis</i> (29212) (00087*)	≥10 ³	inhibited	0%	—

Key : * = corresponding WDCM Numbers
▲ : Formerly known as *Enterobacter aerogenes*
: Formerly known as *Enterobacter sakazakii*

Storage and Shelf-life

Store between 2-8°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. Use before expiry date on the label.

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 (7, 8).

References

1. International Organization for Standardization Draft ISO/ TS 22964, 2006 (E).
2. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1, American Society for Microbiology, Washington, D. C.
3. Muytjens H. L., Zanen H. C., Sonderkamp H. J. et al, J. Clin Microbiol 18:115-120,1983.
4. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
5. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
7. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
8. 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.

Ready Prepared Media

Code	Product Name	Usage	Packing
Category : HiTouch™ FlexiPlate™			
FL036	HiTouch™- HiCrome Ent. Sakazakii Agar Flexi Plate™	for enumeration (count) and differentiation of <i>Enterobacter sakazakii</i> (<i>Cronobacter sakazakii</i>).	50 plts