



HardyCHROM™ Sakazakii

Cat. no. G315	HardyCHROM™ Sakazakii, 15x100mm Plate, 18ml	10 plates/bag
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INTENDED USE

HardyCHROM™ Sakazakii is a chromogenic medium recommended for the selective isolation and differentiation of *Cronobacter (Enterobacter) sakazakii* from other members of the family *Enterobacteriaceae* based on colony color.

SUMMARY

HardyCHROM™ Sakazakii facilitates the isolation and differentiation of *C. sakazakii* from other members of the family *Enterobacteriaceae*. *C. sakazakii* is a gram-negative, rod-shaped opportunistic pathogen that is associated with a rare, but life-threatening form of meningitis and necrotizing enterocolitis in neonates. The source of infection has been linked to the ingestion of powdered milk-based infant formula intrinsically contaminated by *C. sakazakii*. The organism is both thermotolerant and resistant to dessication, which enables it to survive manufacturing processes.^(1,2)

C. sakazakii produces smooth, bluish-green colonies on HardyCHROM™ Sakazakii as a result of unique bacterial enzyme interactions with chromogenic substances. Other members of the family *Enterobacteriaceae* will produce white or colorless colonies with or without black centers. All gram-positive bacteria and yeast will be inhibited on this medium.^(1,2)

FORMULA

Ingredients per liter of deionized water:*

Peptones	10.0gm
Yeast Extract	5.0gm
Chromogenic Mixture	2.0gm
Ferric Citrate	0.5gm
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25 degrees C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date applies to the product in its intact packaging when stored as directed.

Refer to the keyword "Storage", in the Hardy Diagnostics' software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for *in vitro* diagnostic use only and is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions". The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious

disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the keyword "Precautions", in the Hardy Diagnostics' software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics' software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE

Clinical Procedure

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.⁽²⁻⁵⁾

Method of Use: The plates should be warmed to room temperature and the agar surface should be dry before inoculating. Inoculate the specimen onto the media as soon as possible after it is received in the laboratory. If the material is being cultured from a swab, roll the swab over a small area of the agar surface and streak for isolation. Incubate plates aerobically at 35-37 degrees C. Observe plates for characteristic colonial morphology and color at 24 hours. If negative for *C. sakazakii*, reincubate for an additional 24 hours and read again.

Powdered Infant Formula Procedure

Specimen Collection: Consult listed references for information on specimen collection and processing of food, dairy, water samples and other materials of sanitary significance. Plates should be warmed to room temperature and the agar surface should be dry before inoculating.

Isolation and Enumeration of *C. sakazakii* from Dehydrated Powdered Infant Formula:

1. Sterilize the can lid margins and the spoons used for sampling the cans prior to withdrawing the samples. In triplicate, aseptically weigh 100.0gm of powdered infant formula into individual sterile two liter flasks. Also in triplicate, weigh 10.0gm of infant formula into sterile individual 250ml flasks and 1.0gm of infant formula into sterile individual 125ml flasks.
2. Add nine parts of 45 degrees C. sterile deionized water to each flask to create a 1:10 dilution and gently shake by hand until the powder is in a homogenous suspension. Incubate all of the flasks for 18-24 hours at 35 degrees C.
3. After incubation remove 10ml from each flask and add to a prepared 90ml volume of *Enterobacteriaceae* Enrichment (EE) Broth (Cat. no. U291). Incubate overnight at 35 degrees C.

Spread Plate Method:

1. Aseptically inoculate duplicate plates of HardyCHROM™ Sakazakii with 0.1ml from each incubated enrichment broth.
2. Using a sterile spreader device (Cat. no. 74SM100 or 174CS20), distribute the inoculum evenly over the agar surface. (Note: If the powdered infant formula is suspected to contain high numbers of *C. sakazakii*, the incubated EE broth should be diluted to 10⁻⁴ to 10⁻⁶ with sterile EE broth before plating.)
3. Incubate plates aerobically for 24 hours at 35 degrees C. Do not incubate in CO₂.

Streak Plate Method:

1. Aseptically inoculate duplicate plates of HardyCHROM™ Sakazakii with 10μl of inoculum from each incubated enrichment broth.
2. Inoculate agar surface with 10μl if unoculum from *C. sakazakii* enrichment broth.

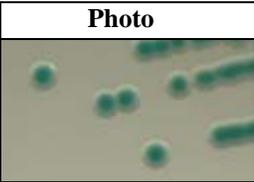
3. Streak plate for isolated colonies.
4. Incubate plates aerobically for 24 hours at 35 degrees C.

Note: It is recommended to do both spread plate and streak plate methods to obtain isolated colonies.

INTERPRETATION OF RESULTS

C. sakazakii produces smooth, bluish-green colored colonies. Other members of the *Enterobacteriaceae* family that can grow on HardyCHROM™ Sakazakii may appear as white or colorless colonies with or without black centers. Gram-positive bacteria and yeast will be inhibited.

Spread Plate Method: Following incubation, examine the plates for growth of *C. sakazakii*. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample; take into account the dilution factor. From the duplicate plates set-up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult listed references for additional information on interpretation and enumeration of microbial growth on this medium.⁽⁶⁻⁸⁾

Organism	Description	Photo	Color
<i>C. sakazakii</i>	bluish-green colonies		
<i>Salmonella</i> spp.	colorless colonies with or without black centers		
<i>Citrobacter</i> spp.	colorless colonies with or without black centers		
<i>Proteus</i> spp.	golden colonies		

LIMITATIONS

It is recommended that biochemical and/or serological tests be performed on colonies from pure culture for complete identification to the species level.

Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ Sakazakii.

Refer to the keyword "Limitations", in the Hardy Diagnostics' software program HUGO™, for more information regarding general limitations on culture media.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, spreaders (Cat. no. 74SM100 or 174CS20), other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Cronobacter sakazakii</i> ATCC® 29544	A	24hr	35°C	Aerobic	Growth; bluish-green colonies

<i>Salmonella enterica</i> ATCC® 14028	A	24hr	35°C	Aerobic	Growth; colorless colonies with black centers
<i>Citrobacter freundii</i> ATCC® 8090	A	24hr	35°C	Aerobic	Growth; colorless colonies with black centers
<i>Proteus mirabilis</i> ATCC® 12453	A	24hr	35°C	Aerobic	Growth; golden colonies
<i>Staphylococcus aureus</i> ATCC® 25923	B	24hr	35°C	Aerobic	Partial to complete inhibition

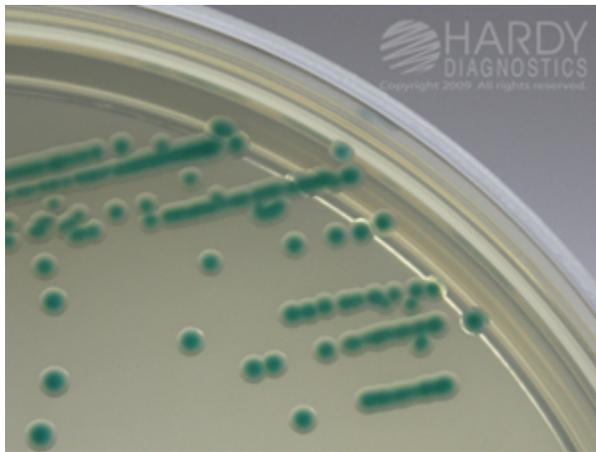
* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics' software program HUGO™, for a description of inoculation procedures.

USER QUALITY CONTROL

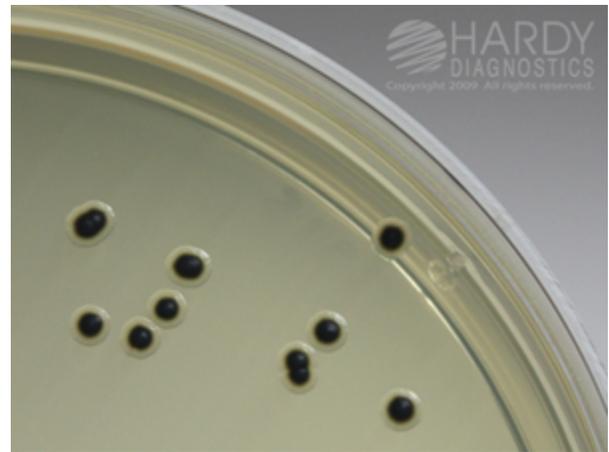
Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). Refer to the following keywords, in the Hardy Diagnostics' software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information.

PHYSICAL APPEARANCE

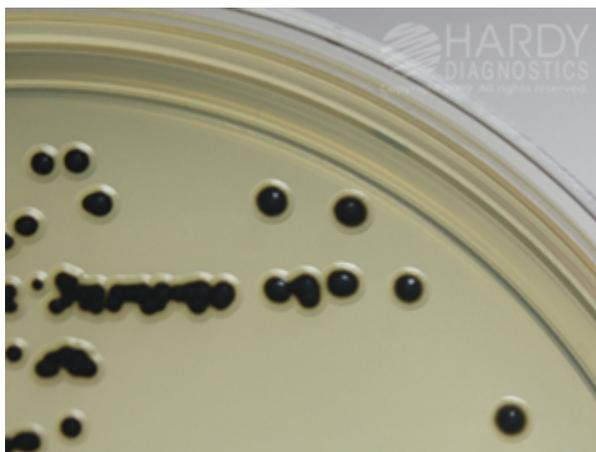
HardyCHROM™ Sakazakii should appear opaque, slightly opalescent, and light amber in color.



Cronobacter sakazakii (ATCC® 29004) colonies growing on HardyCHROM™ Sakazakii (Cat. no. G315). Incubated aerobically for 24 hours at 35 deg. C.



Salmonella enterica (ATCC® 14028) colonies growing on HardyCHROM™ Sakazakii (Cat. no. G315). Incubated aerobically for 24 hours at 35 deg. C.



Citrobacter freundii (ATCC® 8090) colonies growing on HardyCHROM™ Sakazakii (Cat. no. G315).



Proteus mirabilis (ATCC® 12453) colonies growing on HardyCHROM™ Sakazakii (Cat. no. G315).

Incubated aerobically for 24 hours at 35 deg. C.

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REFERENCES

1. Breeuwer, P., et al. 2003. Desiccation and heat tolerance of *Enterobacter sakazakii*. *J. of App. Microbiol.*; 95:967-973.
2. Murray, P.R., *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm>.
6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.

ATCC is a registered trademark of the American Type Culture Collection.

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