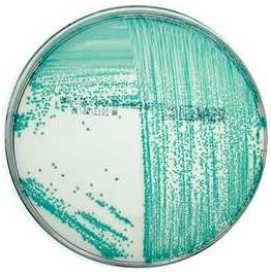
RAPID'*Sakazakii*

Ref#	Description
Pre-poured	
3563971	20 dishes x 90 mm
Dehydrated	
3564976	500 g



FIELD OF APPLICATION

Selective **chromogenic** medium used for the detection of ***Cronobacter* spp.** (formerly ***Enterobacter sakazakii***) in milk powders, dried products for infant feeding and also in their raw materials and their production environment.

PRINCIPLE

The principle of the medium is based on the detection of an enzymatic activity characteristic of *Cronobacter* spp. : α-gluconidase. Under its action, the chromogenic substrate 5-bromo-4-chloro-3-indolyl α-D-glucopyranoside is hydrolyzed inducing a blue to blue green color of *Cronobacter* spp. Colonies.
The incubation temperature set at 44°C, sodium deoxycholate and crystal violet inhibit the growth of some of the associated microflora.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID'*Sakazakii* method has been certified NF VALIDATION as alternative to reference method ISO/TS 22964, according to the EN/ISO 16140:2003 protocol, for the **detection of *Cronobacter* spp.** in infant formula.

BRD 07/22 – 05/12
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

STANDARD REFERENCES

- **ISO/TS 22964:2006 (February 2006)** Milk and milk products - Detection of *Enterobacter sakazakii*

STORAGE / SHELF-LIFE / BATCH

- Deshydrated: **+ 2°** to **8°C**, in carefully sealed bottles in a cool, dry place
- Petri dishes prepared by user: 15 days maximum at + 2° to 8°C, in a dark place
- Expiration date and batch number are shown on the package

THEORETICAL FORMULA

Pancreatic peptone of casein	10,0 g
Yeast extract	3,0g
Sodium chloride	5,0 g
Sodium desoxycholate	0,6g
Crystal violet	2,0 mg
5-bromo-4-chloro-3-indolyl α-D-glucopyranoside	150,0 mg
Agar	18,0 g
Distilled water	1,000 ml

Final pH = 7.2 ± 0.2

OTHER PRODUCTS AND MATERIALS REQUIRED (Not supplied and non-exhaustive list)

- Scales
- Sterile weighing bags
- Mill

- Stirrer-homogeniser
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (0.1 ml ; 1 ml …)
- Sterile spreaders
- Sterile Pasteur pipette
- Water-bath
- Thermostatically-controlled incubators or incubation room
- All usual laboratory equipement

PREPARATION OF DEHYDRATED MEDIUM

Always shake well before use

- Dissolve 36.7 g of powder in 1 litre of distilled water
- Wait 5 min and mix until a homogenous suspension is obtained
- Heat gently, agitating frequently, then boils until completely dissolved
- Dispense, then autoclave at 121 °C (± 1°C) for 15 minutes
- Pour in Petri dishes and leave to dry

Reconstitution ratio : 36.7 g / Liter

PROTOCOL RAPID'*Sakazakii* alternative method Certified NF VALIDATION (short protocol)

- **Sample preparation and incubation**
  - Inoculate η g or η ml of sample on 9 x η ml of buffered peptone water
  - Incubate at 37°C (± 1°C) for 18 h (± 2 h)

- **Isolation and incubation**
  - Take the enrichment broth at the end of the incubation with a sterile loopful
  - Streak onto the surface of RAPID'*Sakazakii*
  - Incubate the upturned dishes at 44°C (± 1°C) for 24 h (± 2 h)
  - Take a reading after 24 h (± 2 h) of incubation

- **Interpretation**
  - Typical colonies of *Cronobacter* spp. are blue to blue-green
  - Non typical colonies are often slightly transparent and purple coloured

- **Confirmation**
  - In the context of NF VALIDATION mark, all samples identified as positive must be confirmed
  - Using the **conventional tests** described in the standardised ISO/TS 22964:2006 reference method including the isolation step on TSA
  - Using iQ-Check® *Cronobacter* PCR reaction (code **3578137)** directly on an isolated colony (with or without purification step)

- **Note:**
  - In the context of NF VALIDATION mark, no samples of over 30 g were tested
  - After incubation, the enrichment broth may be stored at 2°to 8°C for 48 hours, before inoculating RAPID'*Sakazakii*

ISO/TS 22964:2006 protocol

- **Sample preparation, Primary enrichment**
  - Inoculate η g or η ml of sample on 9 x η ml of buffered peptone water
  - Incubate at 37°C (± 1°C) for 18 h (± 2 h)

- **Secondary enrichment**
  - Transfert 0.1 ml of the culture obtain in buffered peptone water in 10 ml of mLST/vancomycine broth.
  - Incubate at 44°C (± 0.5°C) for 24 h (± 2 h)

- **Isolation and incubation**
  - Take the enrichment broth at the end of the incubation with a sterile loopful
  - Streak onto the surface of RAPID'*Sakazakii*
  - Incubate the upturned dishes at 44°C (± 1°C) for 24 h (± 2 h)

- **Confirmation**
  - Using the **conventional tests** described in the standardised ISO/TS 22964:2006 reference method

PRECAUTIONS

- Standard precautions concerning the handling of potentially contaminated products in a microbiology laboratory should be observed. Media that have come in contact with food samples should be considered contaminated and should be autoclaved prior to disposal.
- Comply with Good Laboratory Practice. (EN ISO 7218).
- End of NF VALIDATION: please see the certificate BRD: 07/22–05/12. This certificate is available from Bio-Rad representative or AFNOR Certification.
- See SDS for Product Safety Information, www.bio-rad.com

TECHNICAL SUPPORT IN THE UNITED STATES

In the United States, for technical assistance please call (800) 4BIORAD. Select option 2 for technical support and option 2 again for the food science division. To place an order, please call (800) 4BIORAD and press option 1 for customer care.

QUALITY CONTROL

Every product manufactured and marketed by Bio-Rad is subject to a quality assurance procedure at all stages, from reception of raw materials through to marketing of the finished products. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria. Documentation relative to the production and quality control of each batch is kept on file.

QUALITY AND PERFORMANCE OF THE TEST

See quality certificate available on www.bio-rad.com/certificate (Catalog#/ref# and Lot# number are required)

KEY WORDS

RAPID'*Sakazakii* / *Cronobacter* / *sakazakii* / Detection / Infant Food / Chromogenic / Medium.

BIBLIOGRAPHY

GUILLAUME-GENTIL, O., SONNARD, V., KANDHAI, M.C., MARUGG, J.D., and JOOSTEN, H. 2005.
A simple and rapid cultural method for detection of *Enterobacter sakazakii* in environmental samples. Journal of Food Protection, 68(1) : 64-69.

GURTLER, J.B., KORNACKI, J.L., and BEUCHAT, L.R. 2005.
Enterobacter sakazakii: a coliform of increased concern to infant health. International Journal of Food Microbiology, 104 : 1-34.

IVERSEN, C., DRUGGAN, P., and FORSYTHE, S. 2004**.**
A selective differential medium for *Enterobacter sakazakii*, a preliminary study. International Journal of Food Microbiology, 96 : 133-139.

LEHNER, A., and STEPHAN, R. 2004.
Microbiological, epidemiological and food safety aspects of *Enterobacter sakazakii*. Journal of Food Protection, 67(12) : 2850-2857.

SIMMONS, B.P., GELFAND, M.S., HASS, M., METTS, L. and FERGUSON, J. 1989.
Enterobacter sakazakii infections in neonates associated with intrinsic contamination of a powdered infant formula. Infection Control and Hospital Epidemiology, 10 : 398-401.

For more information about Bio-Rad Food Testing products, visit our website: **www.foodscience.bio-rad.com**