

Rosenow Cysteine/Broth (with brain and marble)

355-5683
356-4922

DEFINITION

Medium used for the detection and enumeration of *Clostridium* and *Bacillus thermophiles* spores in canned food (Most Probable Number technique).

PRINCIPLE

The principle of the Rosenow Cysteine medium relies on the ability to obtain a rapid and abundant culture of particularly fastidious bacteria, both facultative aero-anaerobes and strict anaerobes.

The cysteine hydrochloride present in the medium also makes it possible to obtain an oxidation reducing potential which is conducive to the growth of anaerobic bacteria.

PRESENTATION

• Ready-to-use (on subscription)

10 ml x 25 tubes

code 355-5683

• Set for 5 liters of medium code 356-4922

1 bottle (105 g) of dehydrated medium
1 bottle of pieces of white marble
1 bottle of freeze-dried brain fragments

STORAGE

- Ready-to-use: +2°C to 8 °C.
- +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	10 g
Meat extract	3 g
Sodium chloride	5 g
Glucose	2 g
Soluble starch	2 g
Cysteine hydrochloride	0.3 g
Andrade indicator (5% fuchsin acid)	10 ml
White marble	1 piece/tube
Freeze-dried brain	1 piece/tube
Distilled water	1,000 ml
Final pH (25°C) = 7.2 ± 0.2	

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED)

(non-exhaustive)

- Scales
- Sterile weighing bags
- Mixer-homogenizer
- Test tubes (16x160 mm) with autoclave-proof stoppers
- Sterile pipettes (1 ml, 10 ml, etc)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use.

Dissolve 21g of powder in 1 liter of distilled water. Mix heating gently and swirling frequently, until completely dissolved.

Dispense 10 ml of medium per 16 x 160 mm tube. Add a piece of marble and a fragment of freeze-dried brain to each tube.

Sterilize in autoclave at 121°C for 20 minutes. Store the medium between 0° and 5°C for a maximum of 2 weeks.

Reconstitution rate: 21 g/l.

500 g of powder makes 23.8 liters of medium.

PROTOCOL

• Preparation of samples

According to the standards applicable to the product concerned.

To detect vegetative forms (thermotolerant *Bacillus* and *Clostridium* spores), a heat selection test is necessary. This is carried out by subjecting the stock suspension to a temperature of 98°C for 30 minutes.

• Inoculation and incubation

After regeneration and cooling of the tubes to between 44°C-47°C, inoculate with 1 ml of stock suspension or of its decimal dilutions (3 tubes/ dilution or stock suspension).

After inoculation, pour pure sterile paraffin or previously-melted white agar into each tube. Incubate the tubes at 55°C (± 1°C).

READING AND INTERPRETATION

• Reading

Observe the tubes every day for 8 days and note which tubes show sign of microbial development (positive tubes):

- indicator turning to scarlet red (acidification) or to yellow/green (alkalinization).
- or/and turbidity
- or/and gas formation

• Expression of results/Calculation

Calculate the number of thermotolerant spores By applying the MPN tables using the tubes which register as positive during the 8 days of incubation (See the corresponding standard).

PRECAUTIONS

- Avoid any re-oxygenation of the base broth after regeneration. The inoculum is mixed with the broth using circular movements, taking care to prevent any air from entering the broth.
- A Vortex-type shaker should not be used.
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains.

STRAINS	Anaerobic culture at 37°C after 24-48 hours
<i>Clostridium perfringens</i> ATCC 13124	+ Reduction
<i>Peptostreptococcus Anaerobius</i> CIP 602	+ Reduction
<i>Bacteroides fragilis fragilis</i> ATCC 25285	+ Acidification

STRAINS	Aerobic culture at 37°C after 24 hours
<i>Streptococcus pyogenes</i> ATCC 19615	+ Acidification
<i>Streptococcus pneumoniae</i> ATCC 6303	+ Acidification

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

Rosenow Cysteine / *Clostridium* / Thermophile spores / Food products / Water / Detection / Enumeration / MPN / Medium.