

BRYANT & BURKEY BROTH WITH RESAZURIN (MODIFIED BERGERE)

ENUMERATION OF SPORES OF *CLOSTRIDIUM TYROBUTYRICUM*

1 INTENDED USE

Bryant and Burkey Broth with Resazurin (modified by Bergère) is used to enumerate the spores of lactate fermenting *Clostridium* in milk and dairy products. In particular, it is used to detect *Clostridium tyrobutyricum*, responsible for the late blowing of cheeses such as gruyère, emmental, gouda, edam, cheddar or parmesan. The phenomenon arises from a high number of spores in the milk used to prepare the cheese. This number depends above all on the type of food given to the animals, since the main source of contamination is silage.

2 HISTORY

Originally described by Bryant and Burkey, the medium was modified by Bergère, who in 1968, carried out a study on the role of silage in butyric contamination of milk and clearly differentiated *Clostridium butyricum* from *Clostridium tyrobutyricum*, the latter being the primary agent in cheese degradation.

3 PRINCIPLES

Tryptone, meat and yeast extracts, and cysteine are the nutritive substrates required for the rapid multiplication of *Clostridium tyrobutyricum* under anaerobic conditions.

Sodium acetate is the principal promoter of spore germination, a process which is also activated by heating to 75°C, which simultaneously destroys the vegetative forms.

Resazurin is a redox indicator and should remain colorless after heating the inoculum in the medium. Any increase in oxygen levels is reflected by the appearance of a pink color. Pink tubes are not to be used when analyzing the results.

The fermentation of lactate in the presence of acetate leads to the gas (hydrogen + carbon dioxide) production. Gas release is visualized with a paraffin plug which is raised.

4 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of medium :

- Tryptone	15,0 g
- Yeast extract	5,0 g
- Meat extract.....	7,5 g
- Cysteine (hydrochloride)	0,5 g
- Sodium lactate	5,0 g
- Sodium acetate	5,0 g
- Resazurin	2,5 mg

pH of the ready-to-use medium at 25°C : 5,9 ± 0,2.

5 PREPARATION

- Suspend 38.0 g of dehydrated medium (BK141) in 1 liter of distilled or deionized water.
- Stir slowly until complete dissolution.
- Dispense 10 mL into 16 x 160 mm tubes.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution :**
38,0 g/L

✓ **Sterilization :**
15 min at 121 °C

6 INSTRUCTIONS FOR USE

- Inoculate with 1 mL of inoculum and each of its tenfold dilutions using the MPN method.
- Pour 2 mL of melted (58-60°C) paraffin, previously sterilized at 121°C for 20 minutes.
- Heat the tubes at 75°C for 15 minutes to destroy vegetative cells and activate spores.
- Cool in an ice-water bath to solidify the paraffin.
- Incubate at 37°C for 7 days.

✓ **Inoculation :**
1 mL/tube MPN

✓ **Incubation :**
7 days at 37°C

NOTE : If after storage the medium is pink (sign of oxidation) for more than 1/3 of its height under the surface, regenerate anaerobic conditions by heating at 100°C for 20 minutes. Do not repeat this operation more than once.

7 RESULTS

Tubes with growth and gas generation, raising the paraffin plug, are considered positive. Enumerate using the most probable number method.

This medium is not selective and only an identification of colonies isolated from positive tubes can be used to confirm the presence of *Clostridium tyrobutyricum*. However, experience has shown that the results obtained with this medium are well correlated with damage occurring in cheese manufacturing.

8 QUALITY CONTROL

Dehydrated medium : cream-white powder, free-flowing and homogeneous.

Prepared medium : amber solution, limpid, with a pink ring on the surface.

Typical culture response after 5 days of anaerobic incubation at 37°C (method MPN) :

Microorganisms	Growth (Productivity Ratio : P_R)	Gas production
<i>Clostridium tyrobutyricum</i> CNRZ 500	$P_R \geq 50 \%$	Positive
<i>Clostridium tyrobutyricum</i> CNRZ 505	$P_R \geq 50 \%$	Positive
<i>Clostridium tyrobutyricum</i> CNRZ 608	$P_R \geq 50 \%$	Positive

9 STORAGE / SHELF LIFE

Dehydrated medium : 2-30 °C.

The expiration date is indicated on the label.

Prepared medium in tubes or vials (*) : 6 months at 2-8°C.

Regenerate 20 minutes at 100°C before inoculating.

(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated medium :

500 g bottle BK141HA
5 kg drum BK141GC

11 BIBLIOGRAPHY

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12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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