ROGOSA AGAR

ENUMERATION OF LACTOBACILLI

1 INTENDED USE

Rogosa Agar is a selective medium used for the enumeration of lactobacilli in meat, food products and biological samples of animal origin.

Given its high concentration in salts, the media is not adapted to the isolation of certain dairy lactobacilli such as *Lactobacillus delbrueckii* subsp. *lactis* or *Lactobacillus delbrueckii* subsp. *bulgaricus*.

2 HISTORY

Rogosa and his coworkers showed that this medium was more selective for lactobacilli than the tomato juice medium previously used. The growth of streptococci, *Proteus* and molds were considerably reduced.

3 PRINCIPLES

In addition to Tryptone and yeast extract rich in B vitamins, Tween 80 is a source of fatty acids required for the development of lactobacilli.

Ammonium citrate and sodium acetate inhibit the development of most contaminants, including streptococci and molds.

The acidic pH resulting from the addition of acetic acid favors the growth of lactobacilli and inhibits that of other bacteria.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :
- Tryptone .... 10,0 g
- Yeast extract .... 5,0 g
- Glucose .... 20,0 g
- Sodium acetate .... 15,0 g
- Ammonium citrate .... 2,0 g
- Monopotassium phosphate .... 6,0 g
- Magnesium sulfate .... 575,0 mg
- Manganese sulfate .... 120,0 mg
- Ferrous sulfate .... 34,0 mg
- Tween 80.. 1,0 g
- Bacteriological agar.. 15,0 g

5 PREPARATION

- Dissolve 74,7 g of dehydrated media (BK033) in 1 liter of distilled or demineralized water.
- Adjust the pH to 5,5 by addition of glacial acetic acid (around 1,3 mL).
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.

> ✓ **Reconstitution :**
> **74,7 g/L**
> **Adjust the pH at 5,5**
> ✓ **Sterilization :**
> **Bring to boil**

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6 INSTRUCTIONS FOR USE

- Transfer 1 mL of the product to analyze and its serial dilutions to Petri plates.
- Pour roughly 15 mL of media per plate.
- Homogenize by swirling and let solidify on a cold, flat surface.
- Place the inoculated plates in an anaerobiosis jar.
- Incubate at 37 °C for 72 hours in an atmosphere enriched with 5-10% CO_2.

> ✓ **Inoculation :**
> **1 mL in pour plates**
>
> ✓ **Incubation :**
> **72 h at 37 °C in 5-10% CO_2 atmosphere**.

7 RESULTS

Lactobacilli produce large, white colonies. Prepare the subcultures required for the biochemical identification of the species found on the plates.

8 QUALITY CONTROL

Dehydrated media : cream-white powder, slightly clumped, brittle.
Prepared media : amber agar.

Typical culture response after 72 hours of incubation in a 5% CO_2 atmosphere at 37 °C :

Microorganisms		Growth
Lactobacillus casei subsp. *rhamnosus*	WDCM 00101	$P_R \geq 70 \%$
Lactobacillus plantarum	ATCC 8014	$P_R \geq 70 \%$
Lactobacillus gasseri	WDCM 00103	$P_R \geq 70 \%$
Escherichia coli	WDCM 00013	Inhibited, score 0
Bacillus cereus	WDCM 00001	Inhibited, score 0

9 STORAGE / SHELF LIFE

Dehydrated media : 2-20 °C.
The expiration date is indicated on the label.

Prepared media in tubes or vials (*) : Not recommended.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK033HA

11 BIBLIOGRAPHY

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Sharpe, M.E. 1960. Selective media for the isolation and enumeration of lactobacilli. Lab. Pract., 9: 223-227.

Sabine, D.B., and Vaselekos, J. 1965. Isolation of *Lactobacillus acidophilus* from fecal material. Nature, 206: 960.

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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