Lactobacillus Bulgaricus Agar Base

Intended Use
Lactobacillus Bulgaricus Agar Base, with acetate buffer used for the isolation and identification of *Lactobacillus bulgaricus*.

Summary
The formuation of Lactobacillus bulgaricus Agar is based on the formulation of the Lactobacilli medium recommended by Kulp and White. APHA proposed a modified formulation of the medium for the isolation and identification of *Lactobacillus bulgaricus* from foods.
Lactobacillus bulgaricus (*Lactobacillus delbrueckii* subsp. *bulgaricus*) is one of several bacteria used for the production of Kisselo mlyako (Bulgarian) - "Sour milk" yoghurt (yogurt). The bacterium was first identified in 1905 by the Bulgarian doctor Stamen Grigorov. It is named after Bulgaria, the country where it was first used (it thrives freely on the Balkan Peninsula). The bacterium feeds on milk and produces lactic acid which also helps to preserve the milk. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are the essential microbial species and are active in symbiotic relationship in yoghurt. Because of the emphasis on maintaining a balance between cocci and rods, techniques are needed to determine the relative proportions of *S. thermophilus* and *L. bulgaricus* when grown together in milk cultures.

Principle
Casein enzymic hydrolysate, yeast extract and beef extract provides nitrogenous, carbonaceous compounds and other essential growth nutrients like vitamin B12-complex. Dextrose is the fermentable carbohydrate source. Polysorbate 80 provides fatty acids required for the metabolism of Lactobacilli. Tomato juice provides an acid environment favour acidophilic bacteria and contains factors which promote the growth of Lactobacilli. Sodium acetate and acetic acid lowers as well the pH and prevents as well swarming of *Lactobacillus bulgaricus*. Dipotassium phosphate and the acetates act as buffering system.

Formula*

Ingredients	**g/L**
Casein enzymic hydrolysate	10.0
Yeast extract	5.0
Beef extract	10.0
Dextrose	20.0
Dipotassium phosphate	2.0
Tomato juice	2.0
Polysorbate 80	1.0
Agar	20.0
Final pH (at 25°C)	6.8 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store below 8°C in tightly closed container, preferably in dessicators and use freshly prepared medium. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 70.00 g of the powder in 920 mL purified / distilled water and heat to boiling to dissolve the powder completely.
2. Add 80 ml Acetate Buffer (11.355% Sodium acetate and 0.99% Acetic acid).
3. Sterilize by autoclaving at 121°C (15psi) for 15 minutes as per validated cycle.
4. DO NOT OVERHEAT THE MEDIUM.
5. Mix well and pour into sterile petridishes.

Quality Control

Dehydrated appearance: Cream to yellow coloured, homogeneous, free flowing powder.
Prepared Appearance: Medium amber coloured, clear to slightly opalescent gel forms in petridishes.
Culture Response: Cultural characteristics observed with added acetate buffer, after an incubation of 18-48 hours at 35-37°C.

Organism (ATCC)	Growth
Lactobacillus bulgaricus (11842)	Good

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Kulp W. L. and White V., 1932, Science, 76:17. 2.
2. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201120010500	Dehydrated Culture Media	500 g