



Sporulation Broth

M1018

Sporulation Broth is used for promoting sporulation in *Bacillus subtilis*.

Composition**

| Ingredients | Gms / Litre |
|--------------------------------|-------------|
| Peptic digest of animal tissue | 6.000 |
| Casein enzymic hydrolysate | 4.000 |
| Yeast extract | 3.000 |
| Beef extract | 1.500 |
| Dextrose | 1.000 |
| Manganous sulphate | 0.300 |
| Final pH (at 25°C) | 6.6±0.2 |

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15.8 grams in 1000 ml distilled water. Mix thoroughly. Heat if necessary to ensure complete solution. Dispense as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 20 minutes.

Principle And Interpretation

Sporulation Broth was originally developed by Arret and Kirshbaum for the detection of antibiotic residues in milk and milk products (1). The medium is specified for the preparation of spores, by APHA, for disc assay procedure for the detection of sulfa drugs and antibiotics in milk (2).

Peptic digest of animal tissue and beef extract provide nitrogen, sulphur and amino acids and essential trace ingredients. Yeast extract is a rich source of vitamin B; dextrose is the energy source. Suspensions containing large numbers of bacterial spores are obtained using Sporulation Broth.

A fresh slant culture of *Bacillus subtilis* is washed with sterile physiological saline onto the surface of Roux bottles containing 300 ml sterile medium. The bottles are incubated at 35°C for 5 days and the resulting growth is suspended into 50 ml of sterile physiological saline. The growth is washed by centrifuging the suspension and discarding the supernatant. The sediment obtained is re-suspended in fresh sterile saline and heated at 70°C for 30 minutes to kill vegetative cells and obtain the spore suspension. This spore suspension can be stored for months for use in detection of penicillin/ antibiotic residues in milk and dairy products (2).

Quality Control

Appearance

Cream to light yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Yellow coloured clear solution without any precipitate

Reaction

Reaction of 1.58% w/v aqueous solution at 25°C. pH : 6.6±0.2

pH

6.40-6.80

Cultural Response

M1018: Cultural characteristics observed after an incubation at 35-37°C for 5 days.

| Organism | Inoculum (CFU) | Growth | Spore formation |
|----------|-------------------|--------|--------------------|
|----------|-------------------|--------|--------------------|

Cultural Response

| | | | |
|---------------------------------------|--------|-----------|----------|
| <i>Bacillus pumilus</i> ATCC 14884 | 50-100 | luxuriant | positive |
| <i>Bacillus subtilis</i> ATCC 6633 | 50-100 | luxuriant | positive |

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Arret B. and Kirshbaum A., 1959, J. Milk and Food Tech., 22:329.
2. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 02 / 2015

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.