# PRODUCT SPECIFICATION SHEET

## Letheen Broth, AOAC (DM315)

**Intended Use**

Letheen Broth, AOAC (DM315) is recommended for evaluating the bactericidal activity of quaternary ammonium compounds, and to determine the suitability of preservatives for use in cosmetic formulations, as specified by the ASTM.

**Product Summary and Explanation**

In 1948, Weber and Black described the value of a highly nutritional solid medium containing lecithin and polysorbates to neutralize the antimicrobial action of quaternary ammonium compounds in sanitizers.[1] In 1965, AOAC accepted this methodology for the antimicrobial assays and extended their use to all the cationic detergents. In 1978, the FDA incorporated it as pre-enrichment medium for every microbial examination of cosmetics.

The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in testing of germicidal activity. Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test. The word Letheen represents a combination of lecithin and polysorbate 80.

Letheen Broth was developed by Quisno, Gibby and Foter[1] by the addition of lecithin and Polysorbate 80 to FDA Broth. Letheen Broth is recommended by AOAC to determine the phenol coefficient of cationic surfactants.[2] Letheen Medium is also recommended for testing of cosmetics.[3]

**Principles of the Procedure**

Letheen Broth, AOAC contains beef extract, casein enzymic hydrolysate, which supplies essential nutrients and other trace elements for the microbial growth. Lecithin and polysorbate 80 enables the recovery of bacteria from solutions containing residues of disinfectant used in sanitization of utensils and equipments. Lecithin neutralizes quaternary ammonium compounds and polysorbate 80 neutralizes phenolic disinfectants, hexachlorophene and formalin. Dehydrated medium may appear moist with brown sugar appearance, which does not indicate deterioration.

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| **Part A** | |
| Peptic digest of animal tissue | 10.00 |
| Beef extract | 5.00 |
| Lecithin | 0.70 |
| Sodium chloride | 5.00 |
| **Part B** | |
| Polysorbate 80 | 5.00 |
| Final pH: 7.0 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

**Precautions**

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

**Directions**

1. Suspend 20.7 grams of part A and 5 grams of part B in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Mix well and dispense as desired.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Part A: Light yellow coloured clear solution in tubes<br>Part B: Yellow coloured solution |
| **Prepared Medium** | Light yellow coloured clear solution in tubes |
| **Reaction of 2.57% Solution** | pH : 7.0 ± 0.2 at 25°C |
| **Gel Strength** | Not Applicable |

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

| Sr. No. | Organisms | Results to be achieved | |
|---|---|---|---|
| | | Inoculum (CFU) | Growth |
| 1. | *Escherichia coli ATCC 25922* | 50 -100 | good-luxuriant |
| 2. | *Staphylococcus aureus ATCC 6538* | 50 -100 | good-luxuriant |
| 3. | *Escherichia coli ATCC 8739* | 50 -100 | good-luxuriant |
| 4. | *Staphylococcus aureus ATCC 25923* | 50 -100 | good-luxuriant |

The organisms listed are the minimum that should be used for quality control testing.

**Test Procedure**
Refer to appropriate references for standard test procedures.
**Results**
Refer to appropriate references and test procedures for interpretation of results.

**Storage**
Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

**Expiration**
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

**Limitations of the Procedure**
1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

**Packaging**
**Product Name Letheen Broth, AOAC**
**Product Code : DM315**
**Available Pack sizes : 500gm**

**References**
1. Weber and Black, 1948, Am. J. Public Health, 38:1405.
2. Horwitz, (Ed.), 2000, Official Methods of Analysis of AOAC International, 17th Ed., Vol.I, AOAC International, Gaithersburg, Mb.
3. American Society for Testing and Materials, 1991, Standard Test Methods for preservatives in water-containing cosmetics, E640-78. Annual Book of ASTM Standards, ASTM, Philadelphia, Pa.

**Further Information**

For further information please contact your local MICROMASTER Representative.

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