

LETHEEN BROTH BASE (7105)

Intended Use

Letheen Broth Base is used with Tween 80 (Polysorbate 80) for the testing of quaternary ammonium compounds for antimicrobial activity.

Product Summary and Explanation

In 1948, Weber and Black described the value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers.¹ The addition of Lecithin and Polysorbate 80 to Tryptone Glucose Extract (TGE) Agar resulted in a medium that effectively neutralizes quaternary ammonium compounds while testing germicidal activity. Letheen Agar is a modification of TGE Agar with the addition of Lecithin and Polysorbate 80.

Letheen Broth Base was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby, and Foter discovered that adding Lecithin and Polysorbate 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts.² The resulting medium, termed “Letheen” (a combination of Lecithin and Tween) was easy to prepare and clear in appearance, aiding to visual inspection for growth. Letheen Broth Base is recommended by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) for use with disinfectants containing cationic surface active materials.³

Letheen Broth Base is specified for use by the American Society for Testing Materials (ASTM) in Standard Test Method for Preservatives in Water-Containing Cosmetics.⁴ Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Principles of the Procedure

Enzymatic Digest of Animal Tissue and Beef Extract provide nitrogen, carbon, vitamins, and minerals in Letheen Broth Base. Sodium Chloride maintains the osmotic balance. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.^{2,5-7}

Formula / Liter

Enzymatic Digest of Animal Tissue.....	10 g
Beef Extract	5 g
Sodium Chloride	5 g
Lecithin.....	0.7 g

Final pH: 7.0 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement / Liter (# 7992)

Tween 80 (Polysorbate 80),
5 mL

Precautions

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

Directions

1. Dissolve 20.7 g of the medium and 5 g of Tween 80 (Polysorbate 80) (# 7992) in one liter of purified water.
2. Heat with frequent agitation to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is gold to amber, clear to moderate haze with none to slight precipitate.

Expected Cultural Response: Cultural response in Letheen Broth Base incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Enterococcus faecalis</i> ATCC® 29212	10 - 300	Good to excellent
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Good to excellent
<i>Pseudomonas aeruginosa</i> ATCC® 27853	10 - 300	Fair to excellent
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 300	Good to excellent
<i>Staphylococcus epidermidis</i> ATCC® 12228	10 - 300	Fair to excellent

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

Test Procedure

Lethen Broth Base is used in a variety of procedures. Consult appropriate references for complete information.^{3,4}

Results

Refer to appropriate references and procedures for results.

Storage

Store 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 by keeping container tightly closed.

Expiration

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

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

Lethen Broth Base	Code No.	7105A	500 g
		7105B	2 kg
		7105C	10 kg
Tween 80 (Polysorbate 80)		7992	100 mL

References

1. Weber, G. R., and L. A. Black. 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24**:134-139.
2. Quisno, R., I. W. Gibby, and M. J. Foter. 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118**:320-323.
3. Association of Official Analytical Chemists. 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, D.C.
4. American Society for Testing Materials. 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards, Philadelphia, PA.
5. Erlandson, A. L., Jr., and C. A. Lawrence. 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science. **118**:274-276.
6. Brummer, B. 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. Appl. Environ. Microbiol. **32**:80-84.
7. Favero (chm.). 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.