

LETHEEN BROTH WITH TWEEN, MODIFIED (7712)

Intended Use

Letheen Broth with Tween, Modified is used for the recovery of microorganisms from cosmetics.

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 in testing of germicidal activity. Total neutralization of disinfectants is critical. Disinfectant residues can result in a false negative (no-growth) test.

Letheen Broth with Tween, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Letheen Broth Base.² Letheen Broth with Tween, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.³ Letheen Broth with Tween, Modified has incorporated Polysorbate 80 (Tween) into the medium.

Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen and carbon, essential for good recovery of a wide variety of bacteria and fungi. The nitrogen level is increased in Letheen Broth, Modified to enhance organism growth. Yeast Extract provides vitamins and cofactors in this medium. Sodium Bisulfite, Polysorbate 80, and Lecithin (present in Letheen Broth Base) neutralizes quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.^{4,5,6,7} These preservatives are commonly used in the cosmetic industry.

Formula / Liter

| | |
|--|--------|
| Letheen Broth Base | 20.7 g |
| Enzymatic Digest of Casein | 5 g |
| Enzymatic Digest of Animal Tissue..... | 10 g |
| Yeast Extract..... | 2 g |
| Sodium Bisulfite | 0.1 g |
| Polysorbate 80 | 5 g |

Final pH: 7.2 ± 0.2 at 25°C

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

Precautions

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

Directions

1. Dissolve 42.8 g of the medium 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, lumpy, and tan.

Prepared Appearance: Prepared medium is light to medium amber, and clear to moderate haze.

Expected Cultural Response: Cultural response in Lethen Broth with Tween, Modified 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 | Growth |
| <i>Escherichia coli</i> ATCC® 25922 | 10 - 300 | Growth |
| <i>Pseudomonas aeruginosa</i> ATCC® 27853 | 10 - 300 | Growth |
| <i>Staphylococcus aureus</i> ATCC® 25923 | 10 - 300 | Growth |

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

Test Procedure³

1. Prepare and dilute samples in Lethen Broth with Tween, Modified in accordance with established guidelines. Proceed with procedure using Lethen Agar Base, Modified, Product No. 7495.
2. Using the spread plate technique, inoculate in duplicate 0.1 mL of the diluted samples onto Lethen Agar Base, Modified, Potato Dextrose Agar containing Chlortetracycline, Baird Parker Agar, Anaerobic Agar, and a second set of Lethen Agar Base, Modified plates.
3. Incubate one set of Lethen Agar Base, Modified at 30°C for 48 hours and the other set at 35°C under anaerobic conditions for 2 - 4 days. Incubate the Potato Dextrose Agar plates at 30°C for 7 days and the Baird Parker Agar plates, if inoculated, at 35°C for 48 hours.
4. Incubate diluted samples from Step 1 at 35°C for 7 days. Subculture enriched samples onto Lethen Agar Base, Modified only if there is no growth on the primary Lethen Agar Base, Modified.

Results

Refer to appropriate references for results, or in Lethen Agar Base, Mod, # 7495, Product Information Sheet.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°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.

Limitation of the Procedure

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

Packaging

| | | | |
|--|-----------------|--------------|--------------|
| Lethen Broth with Tween, Modified | Code No. | 7712A | 500 g |
| | | 7712B | 2 kg |
| | | 7712C | 10 kg |

References

1. **Weber, G. R., and L. A. Black.** 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24**:134-139.
2. **Tomlinson, L. (ed.).** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1992. In Tomlinson, L.A. (ed.). FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
4. **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.
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.