

## LETHEEN BROTH BASE, MODIFIED (7496)

### **Intended Use**

**Letheen Broth Base, Modified** is used with Polysorbate 80 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.<sup>1</sup> 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 Base, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Letheen Broth Base.<sup>2</sup> Letheen Broth Base, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.<sup>3</sup>

### **Principles of the Procedure**

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen and carbon required for good growth of a wide variety of bacteria and fungi. The nitrogen level is increased in Letheen Broth Base, Modified to provide better 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.<sup>4,5,6,7</sup> 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

Final pH: 7.2 ± 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)
5g

### **Precautions**

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

### **Directions**

1. Dissolve 37.8 g of the medium and 5g 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 light beige.

**Prepared Appearance:** Prepared medium is orange to amber and clear to lightly hazy.

**Expected Cultural Response:** Cultural response in Lethen Broth Base, 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 Procedure<sup>3</sup>

1. Prepare and dilute samples in Lethen Broth Base, 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, Product Information Sheet, 7495.

### 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.

### 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 Base, Modified	Code No.	7496A	500 g
		7496B	2 kg
		7496C	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. Tomlinson, L. (ed.). 1992. FDA Bacteriological Analytical Manual, 7<sup>th</sup> 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, 7<sup>th</sup> 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.