

LETTEEN AGAR BASE, MODIFIED (7495)

Intended Use

Lethen Agar Base, Modified is used with Tween 80 (Polysorbate 80) for the isolation 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 Tween 80 (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.

Lethen Agar Base, Modified is based on the formula described in FDA Bacteriological Analytical Manual, and a modification of Lethen Agar Base.² Lethen Agar Base, Modified is recommended by the FDA for use in the microbiological testing of cosmetics.³

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 was increased in Lethen Agar Base, Modified to provide better growth. Yeast Extract provides vitamins and cofactors in this medium. Sodium Chloride maintains the osmotic balance. Sodium Bisulfite, Tween 80 (Polysorbate 80), and Lecithin (present in Lethen Agar Base) neutralizes quaternary ammonium compounds. Polysorbate 80 neutralizes phenols, hexachlorophene, formalin, and with Lecithin, ethanol.⁴⁻⁷ These preservatives are commonly used in the cosmetic industry. Agar is the solidifying agent.

Formula / Liter

Lethen Agar Base	25 g
Enzymatic Digest of Casein	10 g
Enzymatic Digest of Animal Tissue.....	10 g
Yeast Extract.....	2 g
Sodium Chloride	5 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), 7 mL

Note: Equivalence for Tween 80 in grams to mL is @ 1:1

Precautions

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

Directions

1. Suspend 52.1 g of the medium and 7 mL of Tween 80 (Polysorbate 80) in one liter of purified water.
2. Heat with frequent agitation and boil for one minute 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 light to medium yellow, and trace to moderately hazy.

Expected Cultural Response: Cultural response on Lethen Agar Base, Modified incubated aerobically at $35 \pm 2^\circ\text{C}$ 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>Salmonella typhimurium</i> ATCC® 14028	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 Base, Modified in accordance with established guidelines.
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

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Lethen Agar Base, Modified and MacConkey Agar (also Baird Parker if used in Step 2).

Storage

Store sealed bottle containing the dehydrated medium at $2 - 30^\circ\text{C}$. 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 Agar Base, Modified	Code No.	7495A	500 g
		7495B	2 kg
		7495C	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*, 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.