ALLIANCE BIO EXPERTISE

Reference: FLWR3039 **A.B.E. - Technical Data Sheet**

Product: # Letheen Modified Broth + Tween + Lecithin

Specification

Liquid medium for the primary recovery of stressed microorganisms in the microbial examination of cosmetics according to FDA and ISO normative.

Presentation

	Packaging Details	Shelf Life	Storage
6 Prepared bottle Bottle 1L with: 900 ± 10 ml	1 box with 6 bottles 1l. Plastic screw cap.	16 months	8-25 ºC

Composition

Composition (g/l):

Proteose peptone	20.0
Tryptone	5.00
Beef extract	5.00
Yeast extract	2.00
Sodium chloride	5.00
Lecithin	10.0
Polysorbate 80	15.0
Sodium bisulfite	0.10

Description /Technique

Description

In the early 40's, Weber and Black recommended the use of lecithin and polysorbates to neutralize the antimicrobial action of the Quaternary Ammonium Compounds (QAC's).
In 1965 the methodology was accepted by the AOAC for antimicrobial assays and extends their use to all the cationic surfactants (detergents). The TAT (Tryptone-Azolectin-Polysorbate) medium, in the Newburger Cosmetic Analysis Manual, (2nd ed., 1977) is similar in composition and uses the AOAC formulation. In 1978 the FDA (Bacteriological Analytical Manual, 5th edition, 1978) incorporated it as a primary presumptive and enrichment medium for all microbial examination of cosmetics.
The present formulation appears in the 8th edition (1998) of the BAM and the notable modification are the inclusion of sodium chloride providing suitable osmotic pressure and an increased amount of peptones and tissue extracts to promote good growth, these transform this medium into a very rich all-purpose medium suitable for neutralizing almost all preservatives presents in samples under examination.
The ISO Technical Committee on Cosmetics (ISO/TC 217) (2006) has also adopted the present formulation as an alternative enrichment medium prior to microbiological examination but ideally Eugon LT100 Broth should be employed for this.

Technique recommended use:

Collect, dilute and prepare samples and volumes as required according to specifications, directives, official standard regulations and/or expected results. Dispense liquid medium in appropiate containers if the original container is of large volume.
Inoculate asseptically the Bottles/tubes with the prepared sample or its dilution.
Incubate the tubes tightly closed aerobically at 30-35ºC for 24-48-72 h
(Incubation times, temperature and sample volumes may vary depending on the sample, on the specifications...)
Read the turbity increase as growth indicator.
This medium can be used to inoculate any confirmatory, secondary medium by streaking methodology or by spiral method; after proper incubation, enumerate all the colonies that have appeared onto the surface of the secondary agar.
Each laboratory must evaluate the results according to their specifications.
Calculate total microbial count per ml of sample by multiplying the average number of colonies per plate by the inverse dilution factor if streaked a diluted sample. Report results as colony forming Unit (CFU's) pe ml or g along with enrichment and secondary media used, incubation time and temperature.

Quality control

Physical/Chemical control

Color : Yellowish-brown pH: 7.1 ± 0.2 at 25ºC

Microbiological control

Prepare Tubes - Inoculate with 100±20 CFU for Growth Promotion or 10^4-10^6 CFU (selectivity).

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Aerobiosis. Incubation at 30-35 ºC. Reading at 18-24h to 72h.

Microorganism	Growth
Ps. aeruginosa ATCC® 9027, WDCM 00026	Good
Staphylococcus aureus ATCC® 6538, WDCM 00032	Good
Bacillus subtilis ATCC® 6633, WDCM 00003	Good

Sterility control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

Bibliography

· ASTM Standard E 640-78 (1991) Test Method for the preservatives in water-containing cosmetics. Philadelphia. PA. USA.
· ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
· FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
· HORWITZ, W. (2000) Official Methods of Analysis. AOAC International. Gaithersburg. MD. USA.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· ISO 16212 Standard (2017) Cosmetics - Microbiology - Enumeration of yeast and mould.
· ISO 21149 Standard (2017) Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria.
· ISO 21150 Standard (2015) Cosmetics - Microbiology - Detection of Escherichia coli.
· ISO 22717 Standard (2015) Cosmetics - Microbiology - Detection of Pseudomonas aeuruginosa.
· ISO 22718 Standard (2015) . Cosmetics - Microbiology - Detection of Staphylococcus aureus.
· LUCAS, I.P. (1977) Microbiological Examination of Cosmetics. Newburger's Manual of Cosmetic Analysis AOAC. Washington.
· US PHARMACOPOEIA (2002) <61> Microbial Limit Tests. 25th ed. US Pharmacopeial Convention. Rockville. MD. USA.
· WEBER, G.R. & L.A. BLACK (1948) Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. 24:134-139.