EUGON BROTH
EUGON LT SUPPLEMENT
EUGON LT SUP BROTH

Dehydrated medium, supplement, ready to use tubes and flasks

1 - Intended use

For the enumeration of microorganisms in cosmetics.

2 - Compositions - typical formulas *

Dehydrated medium (after reconstitution with 1 L of water)

EUGON BROTH

Tryptone	15.0 g
Soy peptone	5.0 g
Sodium chloride	4.0 g
Glucose	5.5 g
Sodium sulphite	0.2 g
L-cystine	0.7 g

Supplement – Flask content (100 mL)

EUGON LT SUPPLEMENT

Sodium laureth sulphate	1.56 g
Egg lecithin	1.00 g
Polysorbate 80	15.00 g
Purified water	85.00 mL

Ready to use flasks and tubes

EUGON LT SUP BROTH

Tryptone	15.00 g
Soy peptone	5.00 g
Sodium chloride	4.00 g
Glucose	5.50 g
Sodium laureth sulphate	1.56 g
Egg lecithin	1.00 g
Polysorbate 80	15.00 g
Sodium sulphite	0.20 g
L-cystine	0.70 g
Purified water	1000 mL

*the formulas may be adjusted and/or supplemented to meet the required performances criteria

3 - Principle of the method and explanation of the procedure

Eugon LT SUP Broth is recommended for the detection and enumeration of aerobic mesophilic bacteria, yeasts and moulds, for the neutralization of preservatives in cosmetic products and for the dispersion of the sample into the liquid medium.
Eugon LT SUP Broth complies with the requirements of the following standards: ISO 16212, ISO 17516, ISO 18415, ISO 18416, ISO 21149, ISO 21150, ISO 22717 and ISO 22718.[1-8]
Eugon LT SUP Broth is prepared with the medium base Eugon Broth supplemented with egg lecithin, sodium laureth sulphate and polysorbate 80.
Tryptone and soy peptone are sources of nitrogen, carbon and vitamins for the microbial growth. Glucose provides carbon and is source of energy. Lecithin and polysorbate 80 are included as neutralising of antimicrobial agents such as phenyl derivatives, aldehydes and quaternary ammonium salts. Sodium laureth sulphate assures a good dispersion of the cosmetics into the liquid medium.

4- Preparation of dehydrated medium

Preparation of Eugon LT SUP Broth

Suspend 30,4 g in 900 mL of cold purified water. Heat to boiling stirring constantly and add the content of one flask of Eugon LT Supplement (100 mL). Distribute in tubes or bottles and sterilize by autoclaving at 121°C for 15 minutes. After autoclaving, gently shake the medium to mix the phases that may have formed.

Preparation of medium for general purposes

Suspend 30,4 g in 1000 mL of cold purified water. Heat to boiling stirring constantly, distribute and sterilize by autoclaving at 121°C for 15 minutes.

5 - Physical characteristics

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Appearance of liquid supplement	pale yellow, limpid
Solution and prepared medium appearance	yellow, opalescent with a light precipitate
Final pH at 20-25 °C	7.0 ± 0.2

6 - Materials provided - packaging

Product	Type	REF	Pack
Eugon Broth	Dehydrated medium	4016432	500 g (16,4 L)
Eugon LT Supplement	Liquid supplement	421540	6 x 100 mL
Eugon LT SUP Broth	Ready-to-use tubes	551583	20 x 9 mL glass tubes, 17x125 mm, flat bottom, aluminium screw-cap. Packaging: cardboard box.
Eugon LT SUP Broth	Ready-to-use flasks	5115832	6 x 90 mL glass flasks with aluminium screw-cap. Packaging: cardboard box.

7 - Materials required but not provided

Sterile microbiological loops or needles, autoclave, incubator and laboratory equipment as required, ancillary culture media such as Tryptic Soy Agar and Sabouraud Dextrose Agar with chloramphenicol.

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8 - SPECIMENS

The specimens consist of cosmetic products. Refer to the current Standards for sample collection and preparation. The medium is not intended for microbiological examination of clinical specimens.

9 - TEST PROCEDURE

Allow tubes and flasks to come to room temperature.
For the detection of microorganisms by enrichment, prepare the initial suspension of the sample (generally 1:10) by adding at least 1 g or 1 mL of the well-mixed product under test into 9 mL of Eugon LT SUP Broth and, if necessary, prepare the decimal dilutions of the initial suspension using the same broth.
Incubate the initial suspension and the decimal dilutions at 32.5°C ± 2.5°C for a minimum of 20 hours and observe the presence of growth. Using a sterile loop, streak an aliquot of the incubated Eugon LT SUP Broth on the surface of a Petri dish with the specific medium for the target organism.
For the enumeration of microorganisms dilute the sample in the broth, generally by 1:10 and perform the successive dilutions in the broth, if necessary. Within 45 minutes, subculture onto the agar intended for the enumeration of the target microorganisms (Tryptic Soy Agar REF 542150 or Sabouraud Dextrose Chloramphenicol Agar REF 542006).
The user is responsible for choosing the appropriate agar media for detection and enumeration of microorganisms, the incubation temperature and time according to the intended use and to the applied ISO Standard.[1-8]

10- READING AND INTERPRETATION

The presence of microorganisms is indicated by the appearance of turbidity into the broth. For the interpretation of the results obtained on the isolation media refer to the instructions for use of the specific agar medium.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control testing in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory.

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of ready-to-use tubes and flasks of Eugon LT SUP Broth and of the raw material used for the production of prepared tubes (dehydrated Eugon Broth REF 401643 supplemented as required), are tested for productivity and for neutralising properties.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of organisms in test tubes and incubating at 32.5°C ± 2.5° for 20 ± 1 hours and recording the highest dilution showing growth in Reference Batch (Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following strains: *B.subtilis* ATCC 6633, *C.albicans* ATCC 10231, *A.brasiliensis* ATCC 16404, *S.aureus* ATCC 6538, *P.aeruginosa* ATCC 9027, *E.coli* ATCC 8739. The productivity index Gr_{RB}-Gr_{TB} for each test strain shall be ≤ 1.
Productivity is also tested by inoculating 10-50 CFU/mL of *P.aeruginosa* ATCC 9027 and *S.aureus* ATCC 6538 in test tubes and incubating at 32.5°C ± 2.5° for 20 ± 1 hours. After a subculture on TSA Agar plates and incubation, the colonies enumeration shall be > 50,000 CFU/mL.
The neutralising properties are tested according to ISO 21149[3] Standard by inoculating Eugon LT SUP Broth with cetrimonium chloride 0.05 mg/mL with *P.aeruginosa* ATCC 9027 and *S.aureus* ATCC 6538.

13 - LIMITATIONS OF THE METHOD

Because of the wide variety of samples examined, it is the user's responsibility to validate this medium for its specific application. The neutralization of the antimicrobial properties of the sample must be verified and validated.

14 - PRECAUTIONS AND WARNINGS

- The products here described are for microbiological control only; they are for professional use and must be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions.
- The products here described are not classified as dangerous according to current European legislation.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- The products here described contain raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that these products do not contain any transmissible pathogen. Therefore, it is recommended that the products be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Each tube and flask are for single use only. Do not transfer or subdivide the tube and flask contents in other containers
- Be careful when opening screw cap tubes and flasks to prevent injury due to breakage of glass
- Ready-to-use tubes, flasks and the supplement are subject to terminal sterilization by autoclaving process.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the tubes or flasks inoculated with samples or microbial strains in accordance with current local legislation.
- The Certificates of Analysis and the Safety Data Sheet are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Dehydrated medium

Upon receipt, store at 10-30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap were damaged or in case of evident deterioration of the powder (colour changes, hardening, presence of large lumps).

Supplement

Upon receipt, store in the original pack at 2-8°C away from direct light. If properly stored, the flasks may be used up to the expiration date. Do not use the product beyond this date. Before use, check the integrity of the screw cap. Do not use the flasks with signs of deterioration (e.g. microbial contamination, atypical colour).

Ready to use tubes and flasks

Upon receipt, store tubes and flasks in their original pack at 2-25°C away from direct light. If properly stored, the tubes and flasks may be used up to the expiration date. Do not use the tubes beyond this date. After opening the box, the tubes and the flasks can be used up to the expiration date. Opened tubes and flasks must be used immediately. Before use, check the integrity of the screw cap. Do not use tubes and flasks with signs of deterioration (e.g. microbial contamination, atypical colour).

16 - REFERENCES

1. ISO 17516 - Cosmetics - Microbiology - Microbiological limits.
2. ISO 18415 - Cosmetics - Microbiology - Detection of specified and non-specified microorganisms.
3. ISO 21149 - Cosmetics - Microbiology - Enumeration and detection of aerobic mesophilic bacteria.
4. ISO 21150 - Cosmetics - Microbiology - Detection of Escherichia coli.
5. ISO 22717 - Cosmetics - Microbiology - Detection of Pseudomonas aeruginosa.
6. ISO 22718 - Cosmetics - Microbiology - Detection of Staphylococcus aureus
7. ISO 18416 - Cosmetics - Microbiology - Detection of Candida albicans.
8. ISO 16212 - Cosmetics - Microbiology - Enumeration of yeast and mould.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Do not reuse	This side up	
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	Fragile

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 1	Modifications of contents and layout	2021/07

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.