

TECHNICAL DATA SHEET

EUGON LT 100 AGAR

ENUMERATION OF MESOPHILIC AEROBES

1 INTENDED USE

Eugon LT 100 agar is used for the enumeration of total mesophilic aerobic microorganisms in cosmetic products with or without preservatives. It enables luxuriant colonies to form in the case of most microorganisms.

The typical composition corresponds to that defined in the standard NF EN ISO 21149.

2 PRINCIPLES

The medium is composed of a mixture of peptones, cystine, glucose and salts which favor the growth of a wide variety of microorganisms.

Sodium chloride maintains osmotic pressure.

Lecithin and Polysorbate 80 (tween) neutralize the antibacterial activity of most antiseptics or preservatives, such as phenolic derivatives, aldehydes and quaternary ammonium salts.

Triton X-100 favors the dispersion of cells and thus improves enumeration.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone	15,0 g
- Papaic digest of soybean meal	5,0 g
- L-cystine	0,7 g
- Glucose	5,5 g
- Sodium chloride	4,0 g
- Sodium sulfite.....	0,2 g
- Lecithin	1,0 g
- Polysorbate 80 (Tween)	5,0 g
- Triton X-100.....	1,0 g
- Bacteriological agar.....	15,0 g

pH of the ready-to-use media at 25 °C : 7,0 ± 0,2.

4 PREPARATION

Preparation of the dehydrated media :

- Dissolve 52,4 g of dehydrated media (BK138) in 1 liter of distilled or demineralized water.
- Slowly bring to a boil, stirring until complete liquefaction.
- Divide into tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- For surface inoculation, pour the molten media into sterile Petri plates and let cool on a cold, flat surface.

✓ **Reconstitution :**
52,4 g/L

✓ **Sterilization :**
15 min at 121°C

Use of ready-to-melt media :

- With the ready-to-use media (BM045), or if the media has been prepared in advance from the dehydrated product as above, melt the medium for the minimum amount of time needed to achieve total liquefaction.
- Cool and maintain the molten media at 44-47 °C.

5 INSTRUCTIONS FOR USE

Surface inoculation :

- On the surface of plates prepared as above, or from pre-poured media (BM170), transfer 0,1 mL of the product to analyze and its serial dilutions.
- Spread for isolation using a sterile loop or spreader.
- Incubate at $32,5 \pm 2,5$ °C for 72 ± 6 hours.

✓ **Inoculation :**
0,1 mL on surface

✓ **Incubation :**
72 ± 6 h at 32,5 ± 2,5 °C

NOTE

It is possible to use membrane filtration for enumeration ; deposit the membrane on the surface of the agar.

Pour plate inoculation :

- Transfer 1 mL of the product to analyze and its serial tenfold dilutions in sterile Petri plates.
- Pour in 15 to 20 mL of the molten medium per plate.
- Homogenize by swirling and let solidify on a cool surface.
- Incubate at $32,5 \pm 2,5$ °C for 72 ± 6 hours.

✓ **Inoculation :**
1 mL in pour plates

✓ **Incubation :**
72 ± 6 h at 32,5 ± 2,5 °C

6 RESULTS

Only count colonies in Petri plates containing less than 300 colonies when using pour plates or surface inoculation. For membrane filtration, only count colonies on membranes with less than 150 colonies.

7 QUALITY CONTROL

Dehydrated media : beige powder, homogeneous, slightly clumped.

Prepared media : amber agar.

Typical culture response after incubation 48 h at 32,5 °C:

Microorganisms	Growth (Productivity Ratio : P_R)
<i>Escherichia coli</i>	WDCM 00012 $P_R \geq 70$ %
<i>Staphylococcus aureus</i>	WDCM 00032 $P_R \geq 70$ %
<i>Bacillus subtilis</i>	WDCM 00003 $P_R \geq 70$ %
<i>Pseudomonas aeruginosa</i>	WDCM 00026 $P_R \geq 70$ %

8 STORAGE / SHELF LIFE

Dehydrated media : 2-20 °C.

Ready-to-melt media in vials : 2-25 °C.

Pre-poured media in Petri plates : 2-8 °C.

The expiration dates are indicated on the labels.

Prepared media in vials or tubes (*) : 180 days at 2-25 °C.

Prepared media in plates (*) : 30 days at 2-8 °C.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media :

500 g bottle BK138HA

Ready-to-melt media :

10 x 200 mL vials BM04508

Pre-poured media in Petri plates (Ø 90 mm) :

20 plates BM17008

10 BIBLIOGRAPHY

Quisno, R., Gibby, I.W., and Fàter, M.J.. 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. *American Journal of Pharmacy*, **118** : 320-323.

Willimson, P., and Kligman, A.M.. 1965. A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology*, **45** : 498-503.

Desbordes, J.. 1977. Biodégradation microbienne des antiseptiques et conservateurs. Conséquences pour la qualité microbiologique. *Revue de l'Institut Pasteur de Lyon*, **10**, n° 4 : 291-311.

Singer, S.. 1987. The use of preservative neutralizers in diluents and plating media. *Cosmetics and Toiletries*, **102** : 55-60.

NF EN ISO 21149. Septembre 2009. Cosmétiques. Microbiologie. Dénombrement et détection des bactéries aérobies mésophiles.

11 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Code document : EUGON LT100 Agar_ENv9
Creation date : 01-2003
Updated : 05-2016
Origin of revision : General update.