B12 Culture Agar(E. coli Maintenance Medium)(E. coli Mutant Culture Agar)

M185

Intended Use:

Recommended for propagation, cultivation and maintenance of *Escherichia coli* mutant used in microbiological assay of Vitamin B_{12}.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	5.000
HL extract #	0.050
Sucrose	12.000
Potassium dihydrogen phosphate	0.500
Magnesium sulphate	0.200
Sodium chloride	0.100
Ferrous sulphate	0.001
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters
Equivalent to Liver extract

Directions

Suspend 37.85 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

B12 Culture Agar (E.coli Mutant Culture Agar) is recommended for cultivation and maintenance of *E.coli* ATCC 11105 (1). Tryptone provides nitrogen and amino acids for the growth of *E. coli* 1133. Yeast extract is vitamin source in the medium while sucrose is carbon and energy source. Potassium salt provides buffering capacity to the medium and sodium, magnesium and ferrous salts control permeability of bacterial cells. HL extract enriches the medium for growth of E. coli as well as inhibits gram positive bacteria.

Stock cultures of *E.coli* ATCC 11105 are prepared by stab cultures are made at least three times in a week. Do not use the culture for preparing assay inoculum if it is over 4 days old. Before using a fresh culture for assay, make at least 10 successive transfers of the culture in 15 days period. Incubate the cultures for 16-24 hours at 35°C but hold constant within ± 0.5°C. After incubation store at 2-8°C.

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1.Do not use the culture for preparing assay inoculum if it is over 4 days old.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Gelling

Firm,comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Yellow coloured slightly opalescent gel forms in tubes as slants.

Reaction

Reaction of 3.79% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Escherichia coli ATCC 11105	50-100	luxuriant	>=70%

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. Kavanagh F., (1972), Analytical Microbiology, Academic Press, New York.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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