



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

M185

B12 Culture Agar ( *E.coli* Maintenance Medium) is recommended for propagation, cultivation and maintenance of *Escherichia coli* mutant 113. 3 Davis ATCC 11105 used in microbiological assay of Vitamin B12.

### Composition\*\*

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	5.000
Liver extract	0.050
Sucrose	12.000
Monopotassium 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

### 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.

### Principle And Interpretation

B12 Culture Agar ( *E.coli* Mutant Culture Agar) is recommended for cultivation and maintenance of *E.coli* ATCC 11105 (1). Casein enzymic hydrolysate provides nitrogen and amino acids for the growth of *E. coli* 113.3. 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. Liver 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.

### 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.

#### Cultural Response

Organism	Inoculum (CFU)	Growth	Recovery
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**Cultural Response**

*Escherichia coli* ATCC 11105      50-100      luxuriant       $\geq 70\%$

**Storage and Shelf Life**

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

**Reference**

1. Kavanagh F., (1972), Analytical Microbiology, Academic Press, New York.

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