



B12 Assay Agar (using *E.coli* mutant Culture) (Harrison et al. M110 Medium)

B12 Assay Agar using *E.coli* Mutant Culture is recommended for the microbiological assay of Vitamin B12 by plate method using *E.coli* mutant 113-3 Davis ATCC 11105 as a test organism.

Composition**

A complete dehydrated medium for microbiological assay of Vitamin B12 contains all essential nutritives except Vitamin B12 for the growth of *E.coli* mutant 113-3 Davis ATCC11105. The addition of B12 in specified increasing concentration gives a growth response, which can be measured with zone reader.

Final pH (at 25°C) 7.2 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 51.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well to distribute slight precipitate evenly. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Generally satisfactory results are obtained with B12 at levels ranging from 0 to 300 ng per ml.

Caution: Over heating or over sterilization will give unsatisfactory results.

Principle And Interpretation

B12 Assay Agar is dehydrated medium devoid of Vitamin B12 but containing all the nutrients essential for the growth of *E. coli* mutant 113-3 Davis ATCC-11105. Incorporation of Vitamin B12 in specified increasing amounts gives a growth response that can be measured by the diameter of the zone of growth around the disc or cup containing Vitamin B12.

For the preparation of Standard, make sterile solutions of Vitamin B12 (Cyanocobalamine Reference Standard). For the determination of Vitamin B12 content of unknown materials the assay sample should be properly diluted and applied similarly as the dilutions of the standards.

Inoculum for the assay is prepared by sub-culturing from a stock culture previously made by stab inoculation. Freshly sub-cultured cells incubated at 35°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for this assay.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Medium amber clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 5.15% w/v aqueous solution at 25°C. pH : 7.2 ± 0.2

pH

7.00-7.40

Cultural Response

Microbiological assay of Vitamin B12 was carried out using *E.coli* mutant 113-3 Davis ATCC 11105 as a test organism. Cultural characteristics observed after an incubation at 35- 37°C for 18-24 hours, good growth was obtained around cups containing Vitamin B12 showing an increase in diameter of zone of growth in proportion the increasing Vit B12 concentration in the cup.

Storage and Shelf Life

Store below 8°C, preferably in desiccator and use freshly prepared medium. Use before expiry date on the label.

Reference

1.Harrison,E., Lees, K.A and Wood, F. (1951) Analyst 76: 696.

Revision : 2 / 2015

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