# B12 Assay Agar, Using *E. coli* Mutant Culture (Harrison et al Medium)

**M110**

## Intended Use:

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 additon 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 purified/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 (1).

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.

## Type of specimen

Isolated microorganisms

## Specimen Collection and Handling:

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.

## 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. Freshly prepared plates must be used or it may result in erroneous results.

## 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 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 between 10-30°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle inorder 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.Harrison,E., Lees, K.A and Wood, F. (1951) Analyst 76: 696.
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.

Revision :03/2023