



Vitamin B12 Agar

M417

Intended Use:

Recommended for microbiological assay of Vitamin B12 using *Lactobacillus leichmannii* ATCC 4797 by the cup plate or disc plate method.

Composition**

Ingredients	Gms / Litre
Vitamin free acicase#	10.000
Soya peptone, vitamin free	5.000
Dextrose (Glucose)	20.000
Sodium acetate	12.000
Polysorbate 80 (Tween 80)	1.000
Potassium sulphate	20.000
Dipotassium hydrogen phosphate	1.000
Potassium dihydrogen phosphate	1.000
Magnesium sulphate	0.400
Sodium chloride	0.020
Ferrous sulphate	0.020
Manganese sulphate	0.020
Ribonucleic acid	1.000
Sodium thioglycollate	1.700
L-Cystine	0.200
Adenine sulphate	0.0176
Guanine hydrochloride	0.0124
Uracil	0.010
Xanthine (sodium)	0.010
Folic acid	0.001
Riboflavin (Vitamin B2)	0.002
Thiamine hydrochloride	0.002
Calcium pantothenate	0.002
Niacin	0.002
Pyridoxine hydrochloride	0.004
Pyridoxal 5 phosphate	0.004
Biotin	0.000001
DL-Tryptophan	0.200
Agar	15.000
Final pH (at 25°C)	6.2±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Casein acid hydrolysate, vitamin free

Directions

Suspend 88.62 grams in 1000 ml purified / 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

Vitamin B12 Agar is a dehydrated medium devoid of Vitamin B12 containing all the nutrients essential for the growth of *Lactobacillus leichmannii* ATCC 4797. 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 (1,4). Inoculum for the assay is prepared by sub culturing from a stock culture previously made by stab inoculation. Freshly subcultured organisms incubated at 37°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for the assay. The growth response obtained is turbidometrically or acidimetrically measured. A standard curve is plotted with absorbance as a function of the vitamin B12 concentration. The concentration of vitamin B12 in the test sample is

calculated based on the interpretation of the standard curve.

Extreme care should be taken to avoid contamination of media or glassware used for the assay. Detergent-free clean glassware should be used. Even small amount of contamination by foreign material may lead to erroneous results.

The test organism used for inoculating must be cultured and maintained on media recommended for this purpose.

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 subcultured organisms incubated at 37°C for 24 hours, centrifuged, washed and suspended in 10 ml saline are recommended for the assay. The growth response obtained is turbidometrically or acidimetrically measured.

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.
2. Even small amount of contamination by foreign material may lead to 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

Off-white to yellow homogeneous powder having a tendency to form soft lumps, which can be easily broken down to powder form.

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Light amber coloured clear to slightly opalescent gel forms in Petri plates.

Reaction

Reaction of 8.8% w/v aqueous solution at 25°C. pH : 6.2±0.2

pH

6.00-6.40

Growth

Good growth is seen around the cups containing Vitamin B₁₂ where diameter of the zone of growth increases in proportion to the increasing Vitamin B₁₂ concentration in the cup. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.

Cultural Response

Microbiological assay of Vitamin B₁₂ is carried out using *Lactobacillus leichmanii* ATCC 4797, after an incubation at 35-37°C for 18-24 hours.

Storage and Shelf Life

Store between 2-8°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 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. H. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C
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.
4. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopoeial Convention, Rockville, MD.

Revision : 02 / 2019

Disclaimer :

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