Vitamin B12 Agar

M417

Vitamin B12 Agar is used for Microbiological assay of Vitamin B12 by using *Lactobacillus leichmannii* ATCC 4797 by the cup plate or disc plate method.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate, vitamin free	10.000
Soyapeptone, vitamin free	5.000
Dextrose	20.000
Sodium acetate	12.000
Polysorbate 80	1.000
Potassium sulphate	20.000
Monopotassium phosphate	1.000
Dipotassium 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

Directions

Suspend 88.62 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. 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 (1,2).

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.

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
M417: Microbiological assay of Vitamin B12 is carried out using Lactobacillus leichmanii ATCC 4797, after an incubation at 35-37°C for 18-24 hours.

Organism

Storage and Shelf Life

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

Reference

1. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopeial Convention, Rockville, MD.
2. H. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C

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