BS031	VITAMIN B12 AGAR
Formula	
Ingredients :	**gms/lit.**
Casein acid hydrolysate, vitamin free	10.00
Soyapeptone vitamin free	5.00
Dextrose	20.00
Sodium acetate	12.00
Potassium sulphate	20.00
Polysorbate 80	1.00
Monopotassium phosphate	1.00
Dipotassium phosphate	1.00
Magnesium sulphate	0.40
Sodium chloride	0.02
Ferrous sulphate	0.02
Manganese sulphate	0.02
Ribonucleic acid	1.00
Sodium thioglycollate	1.70
L-Cystine	0.20
DL-Tryptophan	0.20
Adenine sulphate	0.0176
Guanine hydrochloride	0.0124
Uracil	0.01
Xanthine (sodium)	0.01
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
Agar	15.00
Final pH (at 25°C) : 6.2 $\pm$ 0.2	
Directions :	
Suspend 88.62 gms. in 1000ml. distilled water. Heat to boiling dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.	
Principle :	
Lactobacillus species grow poorly on non-selective culture media and require special nutrients for their growth. Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media used for the microbiological assay of vitamins are the maintenance media used for carrying the stock culture, the inoculums media for preparation of the inoculums and the assay media for quantiation of the vitamin under test. Vitamin B_{12} Agar is a dehydrated medium devoid of vitamin B12 and containing all the nutrients essential for the growth of Lactobacillus leichmanni ATCC 4797. The growth of L.leichmanni ATCC 4797 on this medium is in proportion to the concentration of vitamin B_{12} standard curve is constructed with known dilutions of vitamin B_{12}standards.Inoculum for the assay is prepared by subculturing from a stock culture previously made by stab inoculation. Freshly subcultured organisms incubated at 37^0C for 24 hours, centrifuged, washed and suspended in 10ml 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 B_{12} concentration of vitamin B_{12} 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.	

Refer disclaimer Overleaf

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QC Tests – (I)Dehydrated Medium					
	Colour :	Off white to yellow			
	Appearance :	Homogeneous Free Flowing powder			
(II)Rehydrated medium					
	pH (post autoclaving/heating) :	6.2 ± 0.2			
	Colour (post autoclaving/heating) :	Light amber			
	Clarity (post autoclaving/heating) :	Slightly opalescent			
(III)Q.C. Test Microbiological					
	Microbiological assay of Vitamin B_{12} is carried out using Lactobacillus leichmannii (4797). After 18-24 hours incubation at 35°C, good growth is obtained 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.				
	MICROORGANISM (ATCC)	GROWTH			
	Lactobacillus leichmannii (ATCC 4797)	good			
Precautions :	1. For Laboratory Use.				
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.				
Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
Use :	For microbiological assay of Vitamin B12 using Lactobacillus leichmanni ATCC 4797 by the cup plate or disc plate method.				
Storage :	Dehydrated medium andprepared medium– Between 2 to 8°C.				
Packing :	500 gm. bottle				

Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
BS031	88.62GM/LIT	1.13L	6.2 ± 0.2	NIL	121^0C/15 MIN

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