PRODUCT SPECIFICATION SHEET

B12 Assay Medium (Using *L. leichmannii*) (Vitamin B12 Assay Medium) (DM269)

Intended Use

B12 Assay Medium (Vitamin B12 Assay Medium) (DM269) is recommended for Vitamin B12 assay using *Lactobacillus leichmannii ATCC 7830* as the test organism.

Product Summary and Explanation

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. Vitamin assay media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose: Maintenance Media for carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose; Inoculum Media to condition the test culture for immediate use; and Assay Media to permit quantification of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Vitamin B12 Assay Medium is a Vitamin B12 free medium containing all other vitamins and nutrients essential for the growth of *Lactobacillus leichmannii ATCC 7830*. It was first described by Capp et al[1] and is recommended by USP[2] and AOAC,[3] using *Lactobacillus leichmannii ATCC 7830* as the test organism. Standard curve is constructed with known dilutions of vitamin B12 standards.[2, 3]

Principles of the Procedure

B12 Assay Medium is a vitamin B12-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis ATCC 7830*. To obtain a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations giving a growth response that can be measured titrimetrically or turbidimetrically.

Formula / Liter

Ingredients	Gms / Liter
Casein acid hydrolysate, vitamin free	15.00
Dextrose	40.00
Asparagine	0.20
Sodium acetate	20.00
Ascorbic acid	4.00
L-Cystine	0.40
DL-Tryptophan	0.40
Adenine sulphate	0.02
Uracil	0.02
Xanthine (Sodium)	0.02
Riboflavin (Vitamin B2)	0.001
Thiamine hydrochloride	0.001
Biotin	0.00001
Niacin	0.002
p-Amino benzoic acid (PABA)	0.002
Calcium pantothenate	0.001
Pyridoxine hydrochloride	0.004

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Pyridoxal hydrochloride	0.004
Pyridoxamine hydrochloride	0.0008
Folic acid	0.0002
Monopotassium phosphate	1.00
Dipotassium phosphate	1.00
Magnesium sulphate	0.40
Sodium chloride	0.02
Ferrous sulphate	0.02
Manganese sulphate	0.02
Polysorbate 80	2.00
Guanine hydrochloride	0.02
Final pH: 6.1 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. To avoid contamination of media or glassware in microbiological assay procedures great care must be taken. Glassware used should be scrupulously clean and free from detergents and other chemicals.
4. Extremely small amounts of foreign material may be sufficient to give erroneous results.
5. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
6. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions

1. Suspend 8.45 grams of the medium in 100 ml of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Mix well to distribute the slight precipitate evenly.
4. For the assay, dispense 5 ml medium to each assay tube (containing increasing amounts of standard or the unknown). Total volume of 10 ml per tube is adjusted by addition of distilled water.
5. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous having a tendency to form soft lumps which can be easily broken down to powder form
Prepared Medium	Light amber coloured clear solution that may contain a slight precipitate
Reaction of 8.5% solution	pH 6.1 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Microbiological assay of Vitamin B12 is carried out using *Lactobacillus leichmannii ATCC 7830* after an incubation at 35-37°C for 18-24 hours.

Test Procedure

1. Refer assay procedures as outlined in *USP* or AOAC.
2. Use levels of B12 in the preparation of the standard curve according to these references. It is essential that a standard curve be constructed each time an assay is run.

3. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated.
4. Generally satisfactory results are obtained with gradual increase in growth with increasing USP Cyanocobalamin reference standard levels of 0.0, 0.025, 0.050, 0.075, 0.1, 0.125, 0.150 and 0.2 ng per assay tube (10ml) is recorded as equivalent increase in absorbance at 620nm.
5. Stock cultures of *L. delbrueckii* subsp. *lactis* ATCC 7830 are prepared by stab inoculation into 10 mL of B12 Culture Agar or Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the cultures are kept refrigerated.
6. The inoculum for assay is prepared by subculturing a stock culture of *L. delbrueckii* subsp. *lactis* into 10 mL of B12 Inoculum Broth.
7. Refer to *USP,* for a complete discussion on B12 Culture Agar and B12 Inoculum Broth.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these values. Use only those values that do not vary more than ±10% from the average and use the results only if two-thirds of the values do not vary more than ±10%.

Storage

Store the sealed bottle containing the dehydrated medium at 10- 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. For successful results to these procedures, all conditions of the assay must be followed precisely.
3. Aseptic technique should be used throughout the assay procedure.
4. The use of altered or deficient media may cause mutants having different nutritional requirements and will not give a satisfactory response.

Packaging

Product Name : B12 Assay Medium (Using L. leichmannii) (Vitamin B12 Assay Medium)
Product Code : DM269
Available Pack sizes : 100gm

References

1. Capps B. E., Hobbs M. H. H. and Fox S. H., 1949, J. Biol. Chem., 178:517.
2. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopeial Convention, Rockville, MD.
3. 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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Further Information

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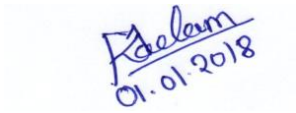		
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