

B₁₂ Assay Medium

Intended Use

B₁₂ Assay Medium is used for determining vitamin B₁₂ concentration by the microbiological assay technique.

Meets *United States Pharmacopeia (USP)* performance specifications.

User Quality Control

Identity Specifications

Difco™ B₁₂ Assay Medium

Dehydrated Appearance: Very light to light beige, homogeneous, with a tendency to clump.

Solution: 4.25% (single strength) solution, soluble in purified water upon boiling for 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.

Prepared Appearance: Very light to light amber, clear, may have a slight precipitate.

Reaction of 4.25%

Solution at 25°C: pH 6.0 ± 0.1

Cultural Response

Difco™ B₁₂ Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 7830 when prepared in single strength and supplemented with cyanocobalamin (vitamin B₁₂). The medium should produce a standard curve when tested with a cyanocobalamin reference standard at 0.0 to 0.25 ng per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-24 hours. Read the percent transmittance using a spectrophotometer at 530 nm.

Summary and Explanation

Vitamin assay media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation 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.

B₁₂ Assay Medium is used in the microbiological assay of vitamin B₁₂ according to the procedures of the Vitamin B₁₂ Activity Assay in the *USP*¹ and the Cobalamin (Vitamin B₁₂ Activity) Assay in the *Official Methods of Analysis of AOAC International* (AOAC).² *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 7830 (*Lactobacillus leichmannii*) is the test organism used in this procedure.

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

Difco™ B₁₂ Assay Medium

Approximate Formula* Per Liter

Vitamin Assay Casamino Acids	15.0	g
Dextrose	40.0	g
Asparagine	0.2	g
Sodium Acetate	20.0	g
Ascorbic Acid	4.0	g
L-Cystine	0.4	g
DL-Tryptophan	0.4	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Xanthine	20.0	mg
Riboflavin	1.0	mg
Thiamine Hydrochloride	1.0	mg
Biotin	10.0	µg
Niacin	2.0	mg
p-Aminobenzoic Acid	2.0	mg
Calcium Pantothenate	1.0	mg
Pyridoxine Hydrochloride	4.0	mg
Pyridoxal Hydrochloride	4.0	mg
Pyridoxamine Hydrochloride	800.0	µg
Folic Acid	200.0	µg
Monopotassium Phosphate	1.0	g
Dipotassium Phosphate	1.0	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20.0	mg
Ferrous Sulfate	20.0	mg
Manganese Sulfate	20.0	mg
Polysorbate 80	2.0	g

*Adjusted and/or supplemented as required to meet performance criteria.

Precautions

Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Directions for Preparation from Dehydrated Product

1. Suspend 8.5 g of the powder in 100 mL of purified water.
2. Heat with frequent agitation and boil for 2-3 minutes to completely dissolve the powder.
3. Dispense in 5 mL amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust the tube volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

Procedure

Follow assay procedures as outlined in *USP*¹ 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. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. Generally satisfactory results are obtained

with B₁₂ at the following levels: 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 and 0.25 ng per assay tube (10 mL).

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. The inoculum for assay is prepared by subculturing a stock culture of *L. delbrueckii* subsp. *lactis* into 10 mL of B₁₂ Inoculum Broth. For a complete discussion on B₁₂ Culture Agar and B₁₂ Inoculum Broth, refer to USP.¹

Expected 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%.

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.

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ B₁₂ Assay Medium

AOAC USP

Cat. No. 245710 Dehydrated – 100 g*

*Store at 2-8°C.