

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 volumes. Use only those values that do not vary more than $\pm 10\%$ from the average and use the results only if two-thirds of the values do not vary more than $\pm 10\%$.

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. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. II. AOAC International, Gaithersburg, Md.

Availability

Difco™ B₁₂ Assay Medium

AOAC USP

Cat. No. 245710 Dehydrated – 100 g*

*Store at 2-8°C.

B₁₂ Culture Agar • B₁₂ Inoculum Broth

Intended Use

These media conform with specifications of *The United States Pharmacopeia (USP)*.

B₁₂ Culture Agar is used for cultivating *Lactobacillus delbrueckii* subsp. *lactis* ATCC™ 7830 used in the Vitamin B₁₂ Activity Assay.

B₁₂ Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 used in the Vitamin B₁₂ Activity Assay.

Summary and Explanation

Vitamin assay media are prepared for use 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.

Lactobacillus species grow poorly on nonselective culture media and require special nutrients. Mickle and Breed² reported the use of tomato juice in culture media for lactobacilli. Kulp,³ while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

B₁₂ Culture Agar is recommended for maintaining stock cultures of *L. delbrueckii* subsp. *lactis* ATCC 7830 (*Lactobacillus leichmannii*) for use in the Vitamin B₁₂ Activity Assay according to the *USP*.¹

B₁₂ Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 in the microbiological assay of vitamin B₁₂ according to the *USP*.¹

Principles of the Procedure

Peptone provides the nitrogen and amino acids in B₁₂ Culture Agar and B₁₂ Inoculum Broth. Yeast extract is the vitamin source in the formulas. Tomato juice is added to create the proper acidic environment. Dextrose is the carbon source, and polysorbate 80 acts as an emulsifier. Dipotassium phosphate acts as the buffering agent in B₁₂ Inoculum Broth, and monopotassium phosphate is the buffering agent in B₁₂ Culture Agar. Agar is the solidifying agent in B₁₂ Culture Agar.

Formulae

Difco™ B₁₂ Culture Agar

Approximate Formula* Per Liter		
Tomato Juice (from 100 mL)	5.0	g
Proteose Peptone No. 3	7.5	g
Yeast Extract	7.5	g
Dextrose	10.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	1.0	g
Agar	14.0	g

Difco™ B₁₂ Inoculum Broth

Approximate Formula* Per Liter		
Tomato Juice (from 100 mL)	5.0	g
Proteose Peptone No. 3	7.5	g
Yeast Extract	7.5	g
Dextrose	10.0	g
Dipotassium Phosphate	2.0	g
Polysorbate 80	0.1	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.

User Quality Control

Identity Specifications

Difco™ B₁₂ Culture Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in purified water upon boiling. Solution is light to medium amber, opalescent when hot, slightly opalescent with flocculent precipitate when cooled.
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Reaction of 4.7% Solution at 25°C:	pH 6.8 ± 0.1

Difco™ B₁₂ Inoculum Broth

Dehydrated Appearance:	Tan, homogeneous, tendency to clump.
Solution:	3.2% solution, soluble in purified water upon boiling. Solution is medium to dark amber, opalescent when hot, clear when cooled to room temperature.
Prepared Appearance:	Medium amber, clear.
Reaction of 3.2% Solution at 25°C:	pH 6.8 ± 0.1

Cultural Response

Difco™ B₁₂ Culture Agar or B₁₂ Inoculum Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 16-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	7830	3 × 10 ² - 10 ³	Good

Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water:
Difco™ B₁₂ Culture Agar – 47 g;
Difco™ B₁₂ Inoculum Broth – 32 g.
Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Dispense 10 mL amounts into tubes.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of vitamin assay methodology, refer to appropriate procedures outlined in the USP.¹

Expected Results

For test results of vitamin assay procedures refer to the USP.¹

Limitations of the Procedure

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

References

- United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.
- Mickle and Breed. 1925. Technical Bulletin 110, NY State Agriculture Ex. Station, Geneva, N.Y.
- Kulp and White. 1932. Science 76:17.

Availability

Difco™ B₁₂ Culture Agar

USP

Cat. No. 254110 Dehydrated – 100 g*

Difco™ B₁₂ Inoculum Broth

USP

Cat. No. 254210 Dehydrated – 100 g*

*Store at 2-8°C.