# **B**<sub>12</sub> Culture Agar • **B**<sub>12</sub> Inoculum Broth

#### **Intended Use**

 $B_{12}$  Culture Agar is used for cultivating *Lactobacillus delbrueckii* subsp. *lactis* ATCC<sup>TM</sup> 7830 used in the Vitamin  $B_{12}$  Activity Assay.

 $B_{12}$  Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 used in the Vitamin  $B_{12}$  Activity Assay.

These media meet *United States Pharmacopeia* (USP) performance specifications.

#### **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

#### **User Quality Control**

Identity Specifica	tions			
Difco <sup>™</sup> B <sub>12</sub> 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™ B., 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, opal- escent when hot, clear when cooled to room temperature.			
Prepared Appearance:	Medium amber, clear.			
Reaction of 3.2%	nH 6.8 + 0.1			

#### Cultural Response Difco™ B<sub>12</sub> Culture Agar or B<sub>12</sub> Inoculum Broth

Prepare the medium per label directions. Inoculate and incubate at  $35 \pm 2^{\circ}$ C for 16-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus delbrueckii	7020	21/102 103	Card
subsp. <i>lactis</i>	/830	3×10 <sup>2</sup> -10 <sup>3</sup>	Good

and require special nutrients. Mickle and Breed<sup>2</sup> reported the use of tomato juice in culture media for lactobacilli. Kulp,<sup>3</sup> while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

 $B_{12}$  Culture Agar is recommended for maintaining stock cultures of *L. delbrueckii* subsp. *lactis* ATCC 7830 (*Lactobacillus leichmannii*) for use in the Vitamin  $B_{12}$  Activity Assay according to the USP.<sup>1</sup>

 $B_{12}$  Inoculum Broth is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 in the microbiological assay of vitamin  $B_{12}$  according to the *USP*.<sup>1</sup>

#### **Principles of the Procedure**

Peptone provides the nitrogen and amino acids in  $B_{12}$  Culture Agar and  $B_{12}$  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  $B_{12}$  Inoculum Broth, and monopotassium phosphate is the buffering agent in  $B_{12}$  Culture Agar. Agar is the solidifying agent in  $B_{12}$  Culture Agar.

#### Formulae

#### Difco<sup>™</sup> B<sub>12</sub> Culture Agar

12		
Approximate Formula* Per Liter		
Tomato Juice (from 100 mL)	5.0	g
Proteose Peptone No. 3	7.5	g
Yeast Extract	7.5	q
Dextrose	10.0	g
Monopotassium Phosphate	2.0	g
Polysorbate 80	1.0	q
Agar		q
5		5

#### Difco<sup>™</sup> B<sub>12</sub> 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.



## Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water: Difco<sup>™</sup> B<sub>12</sub> Culture Agar – 47 g; Difco<sup>™</sup> B<sub>12</sub> Inoculum Broth – 32 g. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- 3. Dispense 10 mL amounts into tubes.
- 4. Autoclave at 121°C for 15 minutes.
- 5. 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*.<sup>1</sup>

## **Expected Results**

For test results of vitamin assay procedures refer to the USP.<sup>1</sup>

# **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 of 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 that will not give a satisfactory response.

#### References

- United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 1-8-08, online. 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<sup>™</sup> B<sub>12</sub> Culture Agar

USP Cat. No. 254110 Dehydrated – 100 g\*

Difco<sup>™</sup> B<sub>12</sub> Inoculum Broth

#### USP

Cat. No. 254210 Dehydrated – 100 g\* \*Store at 2-8°C.

