

Thiamine Assay Medium • Thiamine Assay Medium LV

Intended Use

Thiamine Assay Medium is used for determining thiamine concentration by the microbiological assay technique using *Lactobacillus fermentum* ATCC™ 9338.

Thiamine Assay Medium LV is used for determining thiamine concentration by the microbiological assay technique using *Weissella* (*Lactobacillus*) *viridescens* ATCC™ 12706.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of medium are used for this purpose:

1. Maintenance Medium: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Medium: To condition the test culture for immediate use;
3. Assay Medium: To permit quantitation of the vitamin under test. Assay media contain all factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Thiamine Assay Medium is prepared according to the formula by Sarett and Cheldelin.¹ *Lactobacillus fermentum* ATCC 9338 is used as the test organism in the microbiological assay of thiamine (vitamin B₁).

Thiamine Assay Medium LV, patterned after APT medium, was described by Deibel, Evans and Niven² for the microbiological assay of thiamine using *Lactobacillus viridescens* ATCC 12706.

Nutritional studies by Evans and Niven³ on the heterofermentative lactobacilli that cause greening in cured meat products indicated that thiamine was an essential vitamin for growth of these organisms. Deibel, Evans and Niven⁴ described APT medium for lactobacilli cultivation. They reported that lactobacilli required at least 10 ng thiamine per mL for growth in contrast to 0.2 to 3 ng per mL for thiamine-requiring streptococci, leuconostocs and staphylococci. Further, they suggested that lactobacilli requiring large amounts of thiamine might be employed in microbiological assay procedures. In 1957,² these authors described a medium for the microbiological assay of thiamine using *Lactobacillus* (now *Weissella*) *viridescens* ATCC 12706 as the test organism. This medium is known as Thiamine Assay Medium LV.

Principles of the Procedure

Thiamine Assay Medium and Thiamine Assay Medium LV are free from thiamine, but contain all other nutrients and vitamins essential for the growth of the test organisms. The addition of thiamine in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formulae

Difco™ Thiamine Assay Medium

Approximate Formula* Per Liter	
Thiamine-Free Tryptone.....	22.0 g
Vitamin Assay Casamino Acids.....	5.0 g
Dextrose.....	40.0 g
Sodium Acetate.....	15.0 g
L-Cystine.....	0.2 g
Adenine Sulfate.....	20.0 mg
Guanine Hydrochloride.....	20.0 mg
Uracil.....	20.0 mg
Riboflavin.....	200.0 µg
Calcium Pantothenate.....	200.0 µg
Niacin.....	200.0 µg
Pyridoxine Hydrochloride.....	200.0 µg
p-Aminobenzoic Acid.....	200.0 µg
Folic Acid.....	5.0 µg
Biotin.....	0.8 µg
Dipotassium Phosphate.....	1.0 g
Monopotassium Phosphate.....	1.0 g
Magnesium Sulfate.....	0.4 g
Sodium Chloride.....	20.0 mg
Ferrous Sulfate.....	20.0 mg
Manganese Sulfate.....	20.0 mg

Difco™ Thiamine Assay Medium LV

Approximate Formula* Per Liter	
Thiamine-Free Yeast Extract.....	10.0 g
Thiamine-Free Tryptone.....	20.0 g
Dextrose.....	20.0 g
Sodium Citrate.....	10.0 g
Dipotassium Phosphate.....	10.0 g
Sodium Chloride.....	10.0 g
Magnesium Sulfate.....	1.6 g
Manganese Sulfate.....	0.28 g
Ferrous Sulfate.....	0.08 g
Polysorbate 80.....	2.0 g

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

Precautions

Great care to avoid contamination of media or glassware must be taken 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 the powder in 100 mL of purified water:
Difco™ Thiamine Assay Medium - 8.5 g;
Difco™ Thiamine Assay Medium LV - 8.4 g.
2. Heat with frequent agitation and boil for 2-3 minutes.
3. Dispense in 5 mL amounts into tubes, evenly dispensing the precipitate.
4. Add standard or test samples.
5. Adjust the volume to 10 mL with purified water.
6. Autoclave at 121°C for 5 minutes.

User Quality Control

Identity Specifications

Difco™ Thiamine Assay Medium

Dehydrated Appearance: Beige, homogeneous, tendency to clump.

Solution: 4.25% (single strength) and 8.5% (double strength) solution, soluble in purified water upon boiling 2-3 minutes. 4.25% solution is light amber, clear, may have a slight precipitate.

Prepared Appearance: 4.25% solution is light amber, clear, may have a slight precipitate.

Reaction of 4.25% Solution at 25°C: pH 6.5 ± 0.2

Difco™ Thiamine Assay Medium LV

Dehydrated Appearance: Beige, homogeneous, tendency to clump.

Solution: 4.2% (single strength) and 8.4% (double strength) solution, soluble in purified water upon boiling 2-3 minutes. 4.2% solution is light amber, clear, may have a slight precipitate.

Prepared Appearance: 4.2% solution is light amber, clear, may have a slight precipitate.

Reaction of 4.2% Solution at 25°C: pH 6.0 ± 0.2

Cultural Response

Difco™ Thiamine Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus fermentum* ATCC™ 9338 when prepared in single strength and supplemented with thiamine. The medium should produce a standard curve when tested using a thiamine hydrochloride reference standard at 0.0 to 0.05 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 16-18 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Difco™ Thiamine Assay Medium LV

Prepare the medium per label directions. The medium supports the growth of *Weissella viridescens* ATCC™ 12706 when prepared in single strength and supplemented with thiamine. The medium should produce a standard curve when tested using a thiamine hydrochloride reference standard at 0.0 to 25.0 ng per 10 mL. Incubate tubes with caps loosened at 30 ± 2°C for 16-20 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Procedure

Thiamine Assay Medium

Prepare stock cultures of the test organism, *Lactobacillus fermentum* ATCC 9338, by stab inoculation on Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism in 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 16-18 hours incubation at 35-37°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 mL sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 mL sterile 0.85% NaCl. Add 0.5 mL of this suspension to 100 mL sterile 0.85% NaCl. Use one drop of the resulting suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The tubes for the Thiamine Assay Medium standard curve contain 0.0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04 and 0.05 µg of thiamine hydrochloride per 10 mL tube. The most effective assay range for Thiamine Assay Medium is between 0.005 and 0.03 µg thiamine.

Prepare the stock solution of thiamine required for the preparation of the standard curve in Thiamine Assay Medium as follows:

1. Dissolve 0.1 g of thiamine hydrochloride in 1,000 mL of purified water (100 µg/mL).
2. Add 1 mL of the solution in Step 1 to 99 mL purified water (1 µg/mL).
3. Add 1 mL of the solution in Step 2 to 99 mL purified water to give a final concentration of 10 ng (0.010 µg/mL). Use 0.0, 0.5, 1, 1.5, 2, 3, 4 and 5 mL of this final solution per tube. Prepare fresh stock solution daily.

After 20-24 hours incubation at 35-37°C, *L. fermentum* ATCC 9338 is capable of using the pyrimidine and thiazole moieties of the thiamine molecule. It is essential that the growth response be measured turbidimetrically prior to this time. Incubate the tubes at 35-37°C for 16-18 hours, then place in the refrigerator for 15-30 minutes to stop growth. The growth can then be measured by any suitable nephelometric method.

Thiamine Assay Medium LV

Prepare stock cultures of the test organism, *W. viridescens* ATCC 12706, by stab inoculation on APT Agar or Lactobacilli Agar AOAC. After 24-48 hours incubation at 30 ± 2°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism to 10 mL APT Broth or Lactobacilli Broth AOAC. After 16-20 hours incubation at 30 ± 2°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 mL sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 mL sterile 0.85% NaCl. Add 1 mL of this cell suspension to 100 mL sterile 0.85% NaCl. Use one drop of this suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The standard curve for Thiamine Assay Medium LV is obtained by using thiamine at levels of 0.0, 1, 2.5, 5, 7.5, 10, 15, 20 and 25 ng of thiamine hydrochloride per 10 mL tube. This is obtained by using 0.0, 0.2, 0.5, 1, 1.5, 2, 3, 4 and 5 mL of the standard solution, which contains 5 ng (0.005 µg) thiamine hydrochloride per mL. The most effective assay range is between 2.5 and 20 ng per tube.

The solution for preparing the standard curve for Thiamine

Assay Medium LV may be prepared as follows:

1. Dissolve 50 mg of thiamine hydrochloride in 500 mL purified water (100 µg/mL).
2. Add 1 mL of the solution in Step 1 to 99 mL purified water (1 µg/mL).
3. Add 1 mL of the solution in Step 2 to 199 mL purified water to give a final concentration of 5 ng (0.005 µg) per mL.

Following incubation of *W. viridescens* ATCC 12706 at 30 ± 2°C for 16-20 hours, the growth response is measured turbidimetrically.

Expected Results

Thiamine Assay Medium and Thiamine Assay Medium LV

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. Aseptic technique should be used throughout the microbiological assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements which will not give a satisfactory response.
4. For successful results, all conditions of the assay must be followed exactly.

References

1. Sarett and Cheldelin. 1944. J. Biol. Chem. 155:153.
2. Diebel, Evans and Niven. 1957. Abstr. A68, p. 28. Bacteriol. Proc. 57th Gen. Meet. Soc. Am. Bacteriologists. 1957.
3. Evans and Niven. 1951. J. Bacteriol. 62:599.
4. Diebel, Evans and Niven. 1955. Abstr. G56, p. 48. Bacteriol. Proc. 55th Gen. Meet. Soc. Am. Bacteriologists. 1955.

Availability

Difco™ Thiamine Assay Medium

Cat. No. 232610 Dehydrated – 100 g*

Difco™ Thiamine Assay Medium LV

Cat. No. 280810 Dehydrated – 100 g*

*Store at 2-8°C.