Pyridoxine Y Medium

Intended Use

Pyridoxine Y Medium is used for determining pyridoxine concentration by the microbiological assay technique.

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 optimum growth of the test organism except the single essential vitamin to be determined.

Pyridoxine Y Medium is patterned after the formulation of Campling and Nixon,[1] and modified by Hurley[2] and Parrish, Loy and Kline.[3] This medium is used in the microbiological assay of pyridoxine using *Saccharomyces cerevisiae* ATCC™ 9080 as the test organism.

Principles of the Procedure

Pyridoxine Y Medium is free from pyridoxine, but contains all other nutrients and vitamins essential for the growth of *S. cerevisiae* ATCC 9080. The addition of pyridoxine in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Pyridoxine Y Medium

Approximate Formula* Per Liter		
Dextrose	40.0	g
L-Asparagine	4.0	g
Ammonium Sulfate	4.0	g
Monopotassium Phosphate	3.0	g
Magnesium Sulfate	1.0	g
Calcium Chloride	0.49	g
DL-Methionine	40.0	mg
DL-Tryptophan	40.0	mg
DL-Isoleucine	40.0	mg
DL-Valine	40.0	mg
L-Histidine Hydrochloride	20.0	mg
Riboflavin	20.0	mg
Biotin Salt	8.0	mg
Inositol	5.0	mg
Ferrous Sulfate	500.0	µg
Thiamine Hydrochloride	400.0	µg
Calcium Pantothenate	400.0	µg
Nicotinic Acid	400.0	µg
Boric Acid	200.0	µg
Potassium Iodide	200.0	µg
Ammonium Molybdate	40.0	µg
Manganese Sulfate	80.0	µg
Copper Sulfate	90.0	µg
Zinc Sulfate	80.0	µg

**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 sterilizing and cooling conditions uniform throughout assay.

User Quality Control

Identity Specifications

Difco™ Pyridoxine Y Medium

Dehydrated Appearance:	White to off-white, fine, free-flowing, homogeneous.
Solution:	5.3% (double strength) solution, soluble in purified water upon boiling for 2-3 minutes. Solution 2.65% (single strength) is colorless to very, very light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) colorless to very light amber, clear, may have a slight precipitate.
Reaction of 2.65% Solution at 25°C:	pH 4.4 ± 0.2

Cultural Response

Difco™ Pyridoxine Y Medium

Prepare the medium per label directions. The medium supports the growth of *Saccharomyces cerevisiae* ATCC™ 9080 when prepared in single strength and supplemented with pyridoxine hydrochloride. The medium should produce a standard curve when tested using a pyridoxine hydrochloride reference standard at 0.0 to 10.0 ng per 10 mL. Incubate the tubes with caps loosened at 25-30°C for 22 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Directions for Preparation from Dehydrated Product

1. Suspend 5.3 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 flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 10 mL with purified water.
6. Steam at 100°C for 10 minutes.

Procedure

Stock cultures of *S. cerevisiae* ATCC 9080 are carried on Lactobacilli Agar AOAC. Following incubation at 25-30°C (held constant within ± 0.5°C) for 18-24 hours, store the cultures in the dark at 2-8°C. Prepare fresh slant cultures every week. Do not use stock cultures for preparing the inoculum if more than one week old. Inoculum for assay is prepared by subculturing a stock culture of *S. cerevisiae* ATCC 9080 into a tube (10 mL) of single strength Pyridoxine Y Medium containing 1 ng per mL each of pyridoxal hydrochloride, pyridoxamine dihydrochloride and pyridoxine hydrochloride. After 18-24 hours incubation at 25-30°C (held constant within ± 0.5°C), centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3× with 10 mL sterile 0.85% saline. After the third wash, resuspend in 10 mL sterile single strength medium and adjust to a turbidity of 45-50% transmittance when read on the spectrophotometer at 660 nm.

It is essential that a standard curve be set up for each separate assay. Conditions of steaming and temperature of incubation which influence the standard curve readings cannot always be duplicated. Obtain the standard curve by using pyridoxine hydrochloride at levels of 0, 1, 2, 4, 6, 8 and 10 ng per flask (10 mL).

The concentrations of pyridoxine hydrochloride required for the preparation of the standard curve may be prepared as follows:

1. Dissolve 50 mg dried pyridoxine hydrochloride in about 100 mL of 1 N HCl.
2. Dilute to 500 mL with additional 1 N HCl.
3. Further dilute by adding 2 mL to 998 mL purified water to make a stock solution containing 200 ng pyridoxine hydrochloride per mL. Prepare the stock solution fresh daily.

To make the standard solution, dilute 1 mL of stock solution with 99 mL purified water, to make a solution containing 2 ng pyridoxine hydrochloride per mL. Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL per assay tube.

Following inoculation, incubate the tubes on a shaker (about 100 rpm) at 25-30°C for 22 hours. Steam in the autoclave for 5 minutes at 100°C to stop growth. Measure the growth turbidimetrically using a spectrophotometer at any specific wavelength between 540 and 660 nm.

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

References

1. Campling and Nixon. 1954. J. Physiol. *126*:71.
2. Hurley. 1960. J. Assoc. Off. Agri. Chem. *43*:43.
3. Parrish, Loy and Kline. 1956. J. Assoc. Off. Agri. Chem. *39*:157.

Availability

Difco™ Pyridoxine Y Medium

Cat. No. 295110 Dehydrated – 100 g*

**Store at 2-8°C.*