Niacin Assay Medium

Intended Use

Niacin Assay Medium is used for determining niacin concentration by the microbiological assay technique.

Meets *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.

User Quality Control

Identity Specifications

Difco™ Niacin Assay Medium

Dehydrated Appearance:	Off-white, homogeneous, tendency to clump.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in purified water upon boiling 2-3 minutes. Single strength solution is very light amber, clear, may have a slight precipitate.
Prepared Appearance:	(Single strength) very light amber, clear, may have a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ Niacin Assay Medium

Prepare the medium per label directions. The medium supports the growth of *Lactobacillus plantarum* ATCC™ 8014 when prepared in single strength and supplemented with nicotinic acid. The medium should produce a standard curve when tested using a nicotinic acid reference standard at 0.0 to 0.25 µg per 10 mL. Incubate tubes with caps loosened at 35-37°C for 18-24 hours. Read the percent transmittance using a spectrophotometer at 660 nm.

Niacin Assay Medium is prepared according to the formula described by Snell and Wright,[1] modified by Krehl, Strong and Elvehjem[2] and Barton-Wright.[3] Niacin Assay Medium is used in the microbiological assay of nicotinic acid or nicotinamide (niacin) using *Lactobacillus plantarum* ATCC™ 8014 as the test organism. The medium is specified in assay procedures published in the *USP*[4] and *Official Methods of Analysis of AOAC International* (AOAC).[5]

Principles of the Procedure

Niacin Assay Medium is a dehydrated medium free from nicotinic acid and its analogs but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC™ 8014. The addition of nicotinic acid or its analogs in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Difco™ Niacin Assay Medium

Approximate Formula* Per Liter		
Vitamin Assay Casamino Acids	12.0	g
Dextrose	40.0	g
Sodium Acetate	20.0	g
L-Cystine	0.4	g
DL-Tryptophan	0.2	g
Adenine Sulfate	20.0	mg
Guanine Hydrochloride	20.0	mg
Uracil	20.0	mg
Thiamine Hydrochloride	200.0	µg
Calcium Pantothenate	200.0	µg
Pyridoxine Hydrochloride	400.0	µg
Riboflavin	400.0	µg
p-Aminobenzoic Acid	200.0	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

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

Procedure

Follow assay procedures as outlined in the *USP*[4] and AOAC[5] publications.

Stock cultures of the test organism *L. plantarum* ATCC 8014 are prepared by stab inoculation of Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, the cultures are kept refrigerated. Transfers are made in triplicate at monthly intervals.

The inoculum for assay is prepared by subculturing a stock culture of *L. plantarum* ATCC 8014 into 10 mL of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 18-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant decanted. The cells are washed three times with 10 mL sterile 0.85% saline. After the third wash, the cells are resuspended in 10 mL sterile 0.85% saline and finally diluted 1:100 with 0.85% sterile saline. One drop of this latter suspension is used to inoculate each 10 mL assay tube.

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. The standard curve is obtained by using niacin at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 µg niacin per assay tube (10 mL). Niacin Assay Medium may be used for both turbidimetric and titrimetric analyses. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C. Titrimetric determinations are best made following 72 hours incubation at 35-37°C.

The concentration of niacin required for the preparation of the standard curve may be prepared by dissolving 0.05 g of niacin in 1,000 mL purified water, giving a stock solution of 50 µg per mL. Dilute the stock solution by adding 1 mL to 999 mL purified water (50 ng/mL). Use 0.0, 0.5, 1, 2, 3, 4 and 5 mL of the 50 ng/mL solution per tube. Other standard concentrations may be used provided the standard falls within the limits specified by AOAC.[5]

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. 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 to these procedures, all conditions of the assay must be followed precisely.

References

1. Snell and Wright. 1941. J. Biol. Chem. *13*:675.
2. Krehl, Strong and Elvehjem. 1943. Ind. & Eng. Chem., Ann. Ed. *15*:471.
3. Barton-Wright. 1944. J. Biochem. *38*:314.
4. 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.
5. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.

Availability

Difco™ Niacin Assay Medium

AOAC USP

Cat. No. 232210 Dehydrated – 100 g*

**Store at 2-8°C.*