

Bases / Media Supplements

Technical Information

Niacin Assay Medium

Product Code: DM 1040

Application: - Niacin Assay Medium is used for the microbiological assay of Niacin (Nicotinic Acid) or Niacinamide using *Lactobacillus* plantarum ATCC 8014 as the test organism

Composition**		
Ingredients	Gms / Litre	
Casein enzymic hydrolysate, vitamin free	12.000	
Dextrose	40.000	
Sodium acetate	20.000	
L-Cystine	0.400	
DL-Tryptophan	0.200	
Adenine sulphate	0.020	
Guanine hydrochloride	0.020	
Uracil	0.020	
Thiamine hydrochloride	0.0002	
Calcium pantothenate	0.0002	
Pyridoxine hydrochloride	0.0004	
Riboflavin (Vitamin B2)	0.0004	
p-Amino benzoic acid (PABA)	0.0002	
Biotin	0.000008	
Dipotassium phosphate	1.000	
Monopotassium phosphate	1.000	
Magnesium sulphate	0.400	
Sodium chloride	0.020	
Ferrous sulphate	0.020	
Manganese sulphate	0.020	
Final pH (at 25°C)	6.8±0.2	
**Formula adjusted, standardized to suit performance	parameters	

Principle & Interpretation

Niacin Assay Medium is classified under the category of assay medium of vitamin assay medium. The other two categories of medium essential in vitamin assay are the inoculum media, used for preparation of inoculum to be used in the assay procedure, and maintenance media, used for maintenance of stock cultures used in assay procedures. Niacin Assay Medium is prepared according to the formula described by Snell and Wright (1) and modified by Krehl, Strong and Elvehjam (2) and Barton- Wright (3). This medium is recommended by AOAC (4) and USP (5). Niacin Assay Medium is used for the assay of Niacin (Nicotinamide) employing *Lactobacillus plantarum* ATCC 8014 as the test organism.

Microbiological assay of Niacin is carried out by using *L. plantarum* ATCC 8014 as the test organism. Standard curve is obtained by using USP (5) Niacin reference standard with levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 and 0.25 mcg niacin per assay tube (10 ml). Niacin Assay Medium is used for both turbidimetric and acidimetric analysis. Turbidimetric determination is made after 16-18 hours incubation at 35-37°C. Acidimetric determinations are best made following 72 hours incubation at 35-37°C.





Dehydrated Culture Media Bases / Media Supplements

Niacin Assay Medium is devoid of nicotinic acid but contains all other nutrients and vitamins essential for the cultivation of L. plantarum ATCC 8014. Addition of Niacin (Nicotinic acid) in specified increasing concentrations gives a corresponding growth response that can be measured turbidimetrically or titrimetrically.

Stock cultures of L. plantarum ATCC 8014 are maintained on Lactobacilli Agar, AOAC (DM 1366). Culture for assay is obtained by inoculating Lactobacilli Agar, AOAC (DM1366) and incubating at 35-37°C for 24-48 hours. These cultures are then inoculated into Lactobacilli Broth, AOAC (DM 1367), to prepare the inoculum. Following an incubation at 35-37°C for 18-24 hours, the cells are centrifuged and washed thrice with 0.85% saline. The appropriate dilution of cells so obtained is used to inoculate tubes of Niacin Assay Medium (DM 1040), containing increasing concentrations of Niacin. Using standard Niacin concentration, a standard curve is obtained. This standard is used to extrapolate the unknown niacin concentration. For detailed procedure, refer standard procedures (4, 5).

Extreme care must be taken to avoid contamination of media or glassware used for microbiological assay procedures. Detergent- free clean glassware should be used. Even small amount of contamination by foreign material can lead to erroneous results.

Methodology

Suspend 7.51 grams of dehydrated powder media in 100 ml distilled water. Mix thoroughly & heat if necessary, to dissolve the medium completely. Shake well to distribute the slight precipitate evenly. For the assay, dispense 5 ml medium per assay tube (containing increasing amounts of standard or unknown) and make up the total volume to 10 ml per tube with distilled water. Sterilize by autoclaving at 15 lbs pressure (121°C) for 10 minutes. Cool immediately. Generally satisfactory results are obtained with niacin or niacinamide at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 and 0.25 mcg per assay tube (10 ml).

Quality Control

Appearance

Off-white to yellow homogeneous free flowing powder

Colour and Clarity

Light amber coloured clear solution which may contain a slight precipitate.

Reaction

Reaction of 7.51% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH Range 6.60-7.00

Cultural Response

DM 1040: Microbiological assay of Niacin is carried out using L.plantarum ATCC 8014 after an incubation at 35-37°C for 16-18 hrs.

Cultural Response

Organism

Growth

Cultural Response

Growth

Good growth is obtained. Gradual increase in growth with increasing conc. of Std Niacin-0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 & 0.25 mcg per assay tube is recorded as equivalent increase in absorbance at 620nm

Storage and Shelf Life

Dried Media: Store below 8°C in a tightly closed container and use freshly prepared medium. Use before expiry date on the label. Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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. Williams, (Ed.), 2005, Official Methods of Analysis of the Association of Official Analytical Chemists, 19th Ed., AOAC, Washington, D.C.

5. The United States Pharmacopoeia, 2006, USP29/NF24, The United States Pharmacopoeial Convention, Rockville, MD.





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