PRODUCT SPECIFICATION SHEET

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulphate (DM720)

Intended Use

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulphate (DM720) is recommended for classification of yeasts based on their ability to assimilate nitrogen and carbon compounds.

Product Summary and Explanation

Yeasts are unicellular, eukaryotic, budding cells that are generally round-to-oval or elongate in shape. Principally, they multiply by the production of blastoconidia (buds).[1] Yeast colonies are moist and creamy or glabrous to embranous in texture.[1] Yeasts are considered opportunistic pathogens.[1]

Yeast Nitrogen Base without Amino Acids and Ammonium Sulphate is used for classifying yeasts based on carbohydrate and amino acids requirements. This medium lacks the amino acids, histidine, methionine and tryptophan and also ammonium sulphate. Yeast Nitrogen Base is prepared as per the formulations of Guenter[2], which in turn is modification of Wickerham's formulation.[3] These media are included in many applications for the study of yeasts in molecular genetics.[4, 5]

Principles of the Procedure

Yeast Nitrogen Base without Amino Acids and Ammonium Sulphate contains essential nutrients and vitamins necessary for cultivation of yeasts, except amino acids and a source of nitrogen and carbohydrates. Wickerham used the following nitrogen sources - ammonium sulphate 1.0 gm/l, potassium nitrate 0.78 gm/l, urea 0.46 gm/l, asparagine 1.0 gm/l, peptone (gelatin) 1.32 gm/l. Yeasts grown on rich medium may carry a reserve of nitrogen in the form of proteins that may result in erroneous findings. To avoid this, 2 serial transfers in complete medium are recommended.

Formula / Liter

Ingredients	Gms / Liter
Biotin	0.000002
Calcium pantothenate	0.0004
Folic acid	0.000002
Inositol	0.002
Niacin	0.0004
p-Amino benzoic acid (PABA)	0.0002
Pyridoxine hydrochloride	0.0004
Riboflavin (Vitamin B2)	0.0002
Thiamine hydrochloride	0.0004
Boric acid	0.0005
Copper sulphate	0.00004
Potassium iodide	0.0001
Ferric chloride	0.0002
Manganese sulphate	0.0004
Sodium molybdate	0.0002
Zinc sulphate	0.0004
Monopotassium phosphate	1.00
Magnesium sulphate	0.50
Sodium chloride	0.10
Calcium chloride	0.10
Final pH: 4.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

A. For Carbon Assimilation tests

1. Prepare the broth base in 10X concentration.
2. Dissolve 1.7 grams in 100 ml distilled water.
3. Add 5 grams ammonium sulphate, 10 mg L-histidine, 20 mg DL-methionine and 20 mg DL- ryptophan.
4. Carbon compounds for assimilation test are added in 10X concentration singly or in combination as required.

B. For Nitrogen Assimilation tests

1. Prepare the medium in 10X concentration.
2. Dissolve 1.7 grams in 100 ml distilled water.
3. Add 1 gram dextrose, 1 mg L-histidine, 2 mg DL-methionine and 2 mg DL-tryptophan.
4. Add nitrogen compounds for assimilation test in 10X concentration singly or in combination as required.
5. Wickerham employed the following nitrogen sources : ammonium sulphate 1gm, potassium nitrate 0.78 gm, urea 0.46 gm, asparagine 1 gm, peptone (gelatin) 1.32 gms.

For A and B, filter sterilize the 10X strength solution. Refrigerate and use as needed. Prepare final medium by aseptically pipetting 0.5 ml of the 10X sterile medium into 4.5 ml sterile distilled water. Mix well.

Quality Control Specifications

Dehydrated Appearance	White to cream homogeneous free flowing powder
Prepared Medium	Colourless (at 10X concentration colour of medium is pale yellow) clear solution without any precipitate.
Reaction of 0.17% Solution	pH : 4.5 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 6-7 days.

Sr. No.	Organisms	Results to be achieved	
		Growth (Plain)	Growth w/ Dextrose
1.	*Kloeckera apiculata ATCC 9774*	none-poor	good
2.	*Saccharomyces cerevisiae ATCC 9763*	none-poor	good
3.	*Saccharomyces uvarum ATCC 28098*	none-poor	good

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Inoculate the prepared tubed medium with the test organism.
2. Incubate at 35-37°C for 6-7 days.
3. After incubation (6-7 days and, if necessary, 20-24 days), shake the tubes to suspend growth.
4. Read for growth.

Results

1. Measure the growth turbidimetrically at 660 nm wavelength using a spectrophotometer.
2. Turbidimetric readings on assay tubes should be comparable to the control.

Storage

Store the sealed bottle containing the dehydrated medium at 10- 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Yeasts grown on a rich medium may carry a reserve of nitrogen in the form of protein. Possible errors due to this reserve are eliminated by making two serial transfers in the complete medium.
2. When the first transfer is seven days old, the culture is shaken and one loopful is transferred to a second tube of the complete medium containing the same source of nitrogen. If a positive test is obtained when the second culture is seven days old, the organism being tested assimilates this particular nitrogen source.

3. Consult appropriate texts for detailed information and recommended procedures.

Packaging
Product Name : Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulphate
Product Code : DM720
Available Pack sizes : 100gm

References

1. Warren and Hazen. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. Guenter, Personal communication.
3. Wickerham L. J., 1951, U.S. Dept. Agric. Tech. Bull No. 1029.
4. Sherman, Fink and Hicks. 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
5. Brownstein, Silverman, Little, Burke, Korsmeyer, Schlessinger and Olson. 1989. Science. 244:1348.

Further Information

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