

## YEAST NITROGEN BASE W/O AMINO ACIDS (7709)

### Intended Use

**Yeast Nitrogen Base w/o Amino Acids** is used for classifying yeasts based on amino acid and carbohydrate requirements.

### Product Summary and Explanation

Yeasts are unicellular organisms which reproduce asexually by budding.<sup>1</sup> Yeasts are used industrially to ferment carbohydrates to such products as alcohol and citric acid, and as a spoilage indicator of dairy products.<sup>2</sup>

Yeast Nitrogen Base media have been prepared according to the formulas of Wickerham<sup>3-7</sup> and Burkholder.<sup>8</sup> Their research on the nutritional requirements of yeast resulted in Yeast Nitrogen Base, a well-defined composition of amino acids, nitrogen, vitamins, and salts. Yeast Nitrogen Base w/o Amino Acids is a variation of Yeast Nitrogen Base, and used for selecting yeasts based on amino acid and carbohydrate requirements. Yeast Nitrogen Base w/o Amino Acids, lacks the amino acids Histidine, Methionine, and Tryptophane. In combination with supplement mixtures, this medium provides an excellent source to cultivate and select auxothrophic strains of yeast that require the addition of essential nutrients like amino acids and/or vitamins.

### Principles of the Procedure

Yeast Nitrogen Base w/o Amino Acids contains a Nitrogen Source for nitrogen assimilation. Vitamins and Trace Element Compounds are required for vigorous growth of yeast cells. Salts maintain the osmotic environment of the medium. The addition of a carbon source, i.e., dextrose or galactose, is required.

### Formula / Liter

#### **Nitrogen Sources**

Ammonium Sulfate ..... 5.0 g

#### **Vitamins**

Biotin ..... 2.0 µg  
 Calcium Pantothenate..... 400.0 µg  
 Folic Acid ..... 2.0 µg  
 Inositol..... 2,000.0 µg  
 Nicotinic Acid ..... 400.0 µg  
 p-Aminobenzoic Acid ..... 200.0 µg  
 Pyridoxine HCl ..... 400.0 µg  
 Riboflavin ..... 200.0 µg  
 Thiamine HCl ..... 400.0 µg  
 Citric Acid..... 0.1 g

#### **Trace Elements**

Boric Acid..... 500.0 µg  
 Copper Sulfate ..... 40.0 µg  
 Potassium Iodide ..... 100.0 µg  
 Ferric Chloride ..... 200.0 µg  
 Manganese Sulfate ..... 400.0 µg  
 Sodium Molybdate ..... 200.0 µg  
 Zinc Sulfate ..... 400.0 µg

#### **Salts**

Potassium Phosphate. Monobasic ..... 1.0 g  
 Magnesium Sulfate ..... 0.5 g  
 Sodium Chloride ..... 0.1 g  
 Calcium Chloride..... 0.1 g

Final pH: 5.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

**Precaution**

1. For Laboratory Use.

**Directions**

1. Dissolve 67 g of the medium in one liter of purified water to make a 10X solution.
2. Supplement as desired.
3. DO NOT AUTOCLAVE.
4. Filter sterilize the medium and dispense one mL aliquots into 9 mL of sterile water in sterile capped tubes.

**Quality Control Specifications**

**Dehydrated Appearance:** Powder is homogeneous, free-flowing, and light yellow.

**Prepared Appearance:** Prepared medium is brilliant to clear and colorless.

**Expected Cultural Response:** Cultural response in Yeast Nitrogen Base w/o Amino Acids, prepared with and without 5% Dextrose, 0.02% DL-Methionine, 0.02% DL-Tryptophan, and 0.01% L-Histidine. The organisms listed below were inoculated and then incubated at 25 - 30°C and examined for growth up to 7 days of incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Growth W/O Supplements	Expected Growth W/ Supplements
<i>Kloeckera apiculata</i> ATCC® 9774	10 - 300	None to poor	Good to excellent
<i>Saccharomyces cerevisiae</i> ATCC® 9080	10 - 300	None to poor	Good to excellent

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

**Test Procedure**

1. Mix the solution thoroughly by shaking before inoculation.
2. Inoculate the prepared tubed medium very lightly with the test organism.
3. Incubate inoculated tube at 25°C for up to 7 days.
4. After incubation, shake the tubes to suspend growth.
5. Read for growth.

Refer to appropriate references for Carbon and Nitrogen Assimilation Tests.

**Results**

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

**Storage**

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

**Expiration**

Refer to 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.

**Limitation of the Procedure**

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

- 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. 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.

### Packaging

<b>Yeast Nitrogen Base w/o Amino Acids</b>	<b>Code No. 7709A</b>	<b>500 g</b>
	<b>7709B</b>	<b>2 kg</b>
	<b>7709C</b>	<b>10 kg</b>

### References

- Warren, N. G. and K. C. Hazen.** 1999. *Candida, Cryptococcus*, and other yeasts of medical importance. In P. R. Murray, E. J. Baron, M. A. Tenover, and R. H. Tenover (eds). *Manual of clinical microbiology*, 7<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
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- Wickerham, L. J.** 1951. Taxonomy of yeasts. Technical bulletin No. 1029, US. Dept. Agriculture.
- Wickerham, L. J.** 1939. *J. Tropical Med. Hyg.* **42**:176.
- Wickerham, L. J.** 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. *J. Bacteriol.* **52**:363.
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- Wickerham, L. J.** 1943. *J. Bacteriol.* **46**:501.
- Burkholder, P. R.** 1943. Vitamin deficiencies in yeasts. *Amer. J. Bot.* **30**:206-211.

### Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.