

AZIDE DEXTROSE BROTH (7315)

Intended Use

Azide Dextrose Broth is used in the detection of fecal streptococci in water, sewage, and milk.

Product Summary and Explanation

Roth originated the formula for Azide Dextrose Broth.¹ In a comparative study, Mallmann and Seligmann investigated the detection of streptococci in water and wastewater using Azide Dextrose Broth.² Their work supported the use of this medium in determining the presence of streptococci in water, wastewater, shellfish, and other materials. Azide Dextrose Broth has been used for primary isolation of streptococci from foods and other specimens of sanitary significance as an indication of fecal contamination.^{3,4}

Azide Dextrose Broth is specified in the presumptive test of water and wastewater for fecal streptococci by the Multiple-Tube Technique.⁵

Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract are the carbon, nitrogen, and vitamin sources used for general growth requirements in Azide Dextrose Broth. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits cytochrome oxidase in Gram-negative bacteria.

Group D streptococci grow in the presence of azide, ferment glucose, and cause turbidity.

Formula / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Beef Extract	4.5 g
Dextrose.....	7.5 g
Sodium Chloride	7.5 g
Sodium Azide	0.2 g

Final pH: 7.2 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. HARMFUL. Harmful if swallowed. Irritating to eyes, skin, and respiratory system.

Directions

1. Dissolve 34.7 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is brilliant to clear, yellow or gold to amber, with none to light precipitate.

Expected Cultural Response: Cultural response in Azide Dextrose Broth at 35 ± 2°C and examined for growth after 18 - 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Escherichia coli</i> ATCC® 25922	~ 10 ³	Inhibited
<i>Enterococcus faecalis</i> ATCC® 19433	10 - 300	Growth

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

Test Procedure⁵

1. Inoculate a series of Azide Dextrose Broth tubes with appropriately graduated quantities of sample. Use sample quantities of 10 mL or less. Use double-strength broth for 10 mL inocula. Consult an appropriate reference for suggested sample sizes.⁵
2. Incubate inoculated tubes at 35°C for 20 – 48 hours.
3. Examine each tube for turbidity at the end of 24 ± 2 hours. If no turbidity is evident, reincubate and read again at the end of 48 ± 3 hours.

Results

A positive test is indicated by turbidity (cloudiness) in the broth. A negative test remains clear. Azide Dextrose Broth tubes showing turbidity after 24 – 48 hours incubation must be subjected to the Confirmed Test Procedure. Consult appropriate references for details of the Confirmed Test Procedure⁵ and further identification of *Enterococcus*.^{5,6}

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30. 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 appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Azide Dextrose Broth is used to detect presumptive evidence of fecal contamination. Further biochemical testing must be done for confirmation.
2. For inoculum sizes of 10 mL or larger, use double strength medium to prevent dilution of ingredients.^{5,6}

Packaging

Azide Dextrose Broth	Code No.	7315A	500 g
		7315B	2 kg
		7315C	10 kg

References

1. **Roth.** 1948. Illinois State Health Department.
2. **Mallmann, W. L., and E. B. Seligmann.** 1950. A comparative study of media for the detection of streptococci in water and sewage. Am. J. Public Health. **40**:286.
3. **Larkin, E. P., W. Litsky, and J. E. Fuller.** 1955. Fecal streptococci in frozen foods. I. A bacteriological survey of some commercially frozen foods. Appl. Microbiol. **3**:98.
4. **Splittstoesser, D. F., R. Wright, and G. J. Hucker.** 1961. Studies on media for enumerating enterococci in frozen vegetables. Appl. Microbiol. **9**:303.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greensberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

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.