

PRODUCT SPECIFICATION SHEET



Azide Dextrose Broth (DM027)

Intended Use

Azide Dextrose Broth (DM027) is recommended for selective isolation and detection of *Streptococci* in food, water, sewage and other sewage contaminated material.

Product Summary and Explanation

Enterococci are better indicators of sewage pollution than *Escherichia coli* as are more resistant to chlorine in water. Until 1984, members of the genus *Enterococcus* were classified as Group D Streptococci. Upon genomic DNA analysis, a separate genus status was provided to them.⁽¹⁾ Azide Dextrose Broth is recommended by APHA for enumeration of faecal Streptococci by MPN technique. The formula for Azide Dextrose Broth originated with Rothe at the Illinois State Health Department.⁽²⁾ In a comparative study, Mallmann and Seligmann^(3,4) investigated the detection of streptococci in water and wastewater using Azide Dextrose Broth. Their work supported use of the medium in determining the presence of streptococci in water, wastewater, shellfish and other materials. Azide Dextrose Broth has also been used for primary isolation of streptococci from foodstuffs and other specimens of sanitary significance as an indication of fecal contamination.⁽⁴⁾ Azide Dextrose Broth is specified for use in the presumptive test of water and wastewater for fecal streptococci by the Multiple Tube Technique.⁽⁶⁾ When large volumes of water samples are to be examined, double strength medium is used. Turbidity in tubes indicates presence of Enterococci, however, it should be further confirmed by inoculation in Ethyl Violet Azide Broth (DM413).⁽⁷⁾

Principles of the Procedure

Azide Dextrose Broth is a highly nutritious medium due to the presence of nutrient rich peptone special, beef extract. 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

Ingredients	Gms / Liter
Peptone, special	15.00
Beef extract	4.50
Dextrose	7.50
Sodium chloride	7.50
Sodium azide	0.20
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 only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.
3. Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.

Directions

1. Suspend 34.7 grams of the medium in one liter of distilled water for preparing single strength broth or use 69.4 grams in one liter distilled water for double strength broth.
2. Heat if necessary to dissolve the medium completely.





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3. Dispense into test tubes.
4. Autoclave at 118°C, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Amber coloured clear solution without any precipitate
Reaction of 3.47% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	<i>Escherichia coli</i> ATCC 25922	$\geq 10^3$	inhibited
2.	<i>Enterococcus faecalis</i> ATCC 29212	50 -100	good-luxuriant

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

Test Procedure

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.
2. Incubate inoculated tubes at 35 ± 2°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.
4. Refer to appropriate references for standard test procedures.

Results

1. A positive test is indicated by turbidity (cloudiness) in the broth.
2. A negative test remains clear.
3. All Azide Dextrose Broth tubes showing turbidity after 24- or 48-hours of incubation must be subjected to the Confirmed Test Procedure.
4. Consult appropriate references for details of the Confirmed Test Procedure and further identification of *Enterococcus*.

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





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Packaging

Product Name : Azide Dextrose Broth

Product Code : DM027

Available Pack sizes : 100gm/ 500gm

References

1. Schleider K.H., Kilpper Bolz R., 1984, Int.J.Sys.Bacteriol., 34:31.
2. Rothe. 1948. Illinois State Health Department.
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4. Larkin, Litsky and Fuller. 1955. Appl. Microbiol. 3:98.
5. Edwards S.J., 1933, J. Comp. Path. Therap., 46:2111.
6. Hartman G., 1937, Milchw. Forsch, 18:166.
7. MacFaddin J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical bacteria, Vol.1. Williams & Wilkins, Baltimore, Md.

Further Information

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