Azide Dextrose Broth (Rothe)

Art. No. 02-027

WARNING

H: 3.1.O/4: H302-4.1.C/3: H412

P: P273-P264-P270-P301+P312-P330-P501a

Specification

Medium for the detection and enumeration of enterococci in water.

Formula* in g/L

Meat peptone	10,00
Casein peptone	10,00
Dextrose	5,00
Sodium chloride	5,00
Dipotassium hydrogen phosphate	2,70
Potassium dihydrogen phosphate	2,70
Sodium azide	0,20

Final pH 7,0 ± 0,2 at 25°C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Dissolve 35,6 g in 1 L of distilled water. Heat if necessary to dissolve. Divide into 10 mL volumes and pour into tubes. Sterilize in the autoclave at 121°C for 15 minutes. For double strength medium, dissolve 71,2 g/L.

Description

Azide Dextrose Broth according to Rothe has been widely used since 1948 for the detection of faecal streptococci. It usually provides a higher rate of positive results than similar media. Its efficacy is due to the Sodium Azide, which is both selective for enterococci and inhibitive to the accompanying flora through interference of the electron transport chain. This medium is also used for the primary enrichment of food samples, particularly frozen vegetables.

Technique

Water Samples

Add 10 mL of water to be examined to each of three tubes containing 10 mL of double strength medium. Add 1 mL of sample to an additional three tubes containing 10 mL, of single strength medium. Then add 0.1 mL of water to each of three tubes containing 10 mL of single strength medium. Incubate at 37°C and examine after 24 and 48 h. All tubes which are turbid due to growth will be considered as presumptively positive and will have to be confirmed using EVA Broth (Art. No. 02-028). All tubes which are positive on this second testing should be considered for testing using the Most Probable Number (MPN) count method.

When considering other type of samples, dilute them in 1/4 Ringer's solution or peptone water and then inoculate the tubes as previously described.

In highly contaminated samples, dilutions should be carried out prior to inoculation.

References

- CLESCERI, L., A.E. GREENBERG & E.A. EATON (1998) Standard Methods for the Examination of Water and Wastewater. APHA-AWWA-WEF. 20th ed. Washington.
- DOWNES, F.C. & K. ITO (2001) Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA. Washington.
- GUINEA, SANCHO & PARÉS (1979) Análisis Microbiológico de Aguas: Aspectos Aplicados. Ed. Omega. Barcelona.
- ISO/TS 11133-1: 2009. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- ISO/TS 11133-2: 2003 Corr. 2004. Microbiology of food and animal feeding stuffs.- Guidelines on preparation and production of culture media. Part 2: Practical guidelines on performance testing of culture media.
- ROTHE (1948) Illinois State Health Department.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4°C to 30°C and <60% RH).

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Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 24 - 48 h

Inoculum: 10-100 CFU (Productivity) // 1.000-10.000 CFU (Selectivity). (ISO/TS 11133-1/2)

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC 25923	Inhibited	-
Escherichia coli ATCC 25922	Inhibited	-
Enterococcus faecalis ATCC 29212	Good to very good	-
Enterococcus faecalis ATCC 19433	Good to very good	-

Left: Uninoculated tube (Control)
Centre: *Enterococcus faecalis* ATCC 29212
Right: *Escherichia coli* ATCC 25922

www.scharlab.com
scharlab@scharlab.com