PRODUCT SPECIFICATION SHEET

Azide Blood Agar Base (DM026)

Intended Use

Azide Blood Agar Base (DM026) is recommended for selective isolation and cultivation of *Staphylococcus* and *Streptococcus* species from mixed bacterial flora.

Product Summary and Explanation

In 1933, Edwards used a liquid medium containing crystal violet and sodium azide as a selective broth in the isolation of Streptococci from bovine mastitis cases.[1] Snyder and Lichstein[2,3] reported that 0.01% sodium azide in blood agar prevented the swarming of *Proteus* species, and permitted the isolation of streptococci from mixed bacterial populations. Packer[4] modified Edwards' medium and prepared Infusion Blood Agar containing 1:15,000 sodium azide and 1:500,000 crystal violet for the study of bovine mastitis. Mallmann, Botwright and Churchil,[5] reported that sodium azide exerted a bacteriostatic effect on gram-negative bacteria. Azide Blood Agar Base is recommended for the isolation and cultivation of *Streptococcus* species from clinical and nonclinical specimens. Azide Blood Agar Base can be supplemented with 5-10% sheep, rabbit or horse blood for isolating, cultivating and determining hemolytic reactions of fastidious pathogens.

Principles of the Procedure

Azide Blood Agar Base contains peptone special and beef extract which are the sources of carbon, nitrogen, amino acids and essential growth factors. Sodium azide acts as a selective agent by suppressing the growth of gram-negative bacteria. It also prevents the swarming of *Proteus*. Sodium chloride helps to maintain the osmotic balance of the medium. The media can be supplemented with sterile defibrinated blood to prepare blood agar. Blood serves as an additional source of growth factors and it also helps to visualize the haemolytic pattern. The pH of the medium influences the inhibitory action of sodium azide. At pH 7.2, sodium azide does not interfere with the haemolytic reactions of *Streptococci;* however, haemolytic pattern of Streptococci is different on Azide Blood Agar as compared on nonselective blood agar.

Formula / Liter

Ingredients	Gms / Liter
Peptone, special	10.00
Beef extract	3.00
Sodium chloride	5.00
Sodium azide	0.20
Agar	15.00
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.

PRODUCT SPECIFICATION SHEET

Directions

1. Suspend 33.2 grams in 1000 ml of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C. For preparing Blood Agar plates, 5% v/v sterile defibrinated blood is added aseptically.
5. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Yellow coloured, clear to slightly opalescent gel After addition of 5%w/v sterile defibrinated blood : Cherry red coloured, opaque gel forms in Petri plates, which darkens on standing
Reaction of 3.32% Solution	pH : 7.2 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response : Cultural characteristics observed with added 5%w/v sterile defibrinated blood, after an incubation at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Haemolysis
1.	*Enterococcus faecalis ATCC 29212*	50-100	good- luxuriant	>=50%	alpha/gamma
2.	*Escherichia coli ATCC 25922*	50-100	none-poor	<=10%	none
3.	*Staphylococcus epidermidis ATCC 12228*	50-100	good- luxuriant	>=50%	none
4.	*Streptococcus pyogenes ATCC 19615*	50-100	good- luxuriant	>=50%	beta
5.	*Streptococcus pneumonia ATCC 6303*	50-100	good- luxuriant	>=50%	alpha

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

Test Procedure

1. For best results, use light inoculum and incubate anaerobically for enhancement in haemolytic reaction.
2. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions demonstrating both oxygen-stable and oxygen-labile streptolysins.
3. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 in accordance with established laboratory procedures.

Results

Examine plates for growth and hemolytic reactions after 18-24 and 40-48 hours of incubation. Four different types of hemolysis on blood agar media can be described:

1. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.

PRODUCT SPECIFICATION SHEET

2. Beta (ß)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
3. Gamma(γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.
4. Alpha-prime (α') hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

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 Blood Agar Base is intended for selective use and should be inoculated in parallel with nonselective media.
2. Hemolytic patterns of streptococci grown on Azide Blood Agar Base are somewhat different than those observed on ordinary blood agar. Sodium azide enhances hemolysis. Alpha and beta zones may be extended.
3. Hemolytic patterns may vary with the source of animal blood or base medium used.
4. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Azide Blood Agar Base
Product Code : DM026
Available Pack sizes : 500gm

References

1. Edwards. 1933. J. Comp. Pathol. Therap. *46*:211.
2. Snyder and Lichstein. 1940. J. Infect. Dis. *67*:113.
3. Lichstein and Snyder. 1941. J. Bacteriol. *42*:653.
4. Packer. 1943. J. Infect. Dis. *67*:113.
5. Mallmann, Botwright and Churchill. 1943. J. Bacteriol. *46*:343.

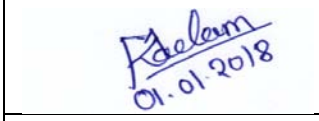
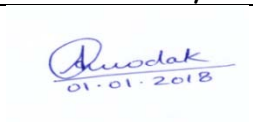
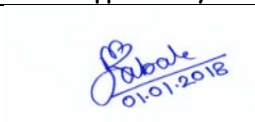
Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,
Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

DM026PSS,QAD/FR/024,Rev.00/01.01.2018

PRODUCT SPECIFICATION SHEET

	Checked By	Approved By
		
Microbiologist	**Head Quality Control**	**Head Quality Assurance**

Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.