TRYPTOSE BLOOD AGAR BASE (7282)

Intended Use

Tryptose Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms.

Product Summary and Explanation

Investigations of the nutritive properties of tryptose found culture media prepared with this peptone were superior to meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, and meningococci. Casman reported that a medium consisting of 2% tryptose, 0.3% beef extract, 0.5% sodium chloride, 1.5% agar, and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms.[1,2]

Tryptose Blood Agar Base is a nutritious, infusion-free basal medium typically supplemented with 5 - 10% sheep, rabbit, or horse blood. This medium is typically used for isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, this blood agar base can be used as a general purpose medium. Tryptose BAB is specified in standard methods for food testing.[3]

Principles of the Procedure

The nitrogen, vitamin, and carbon sources are provided by Tryptose and Beef Extract in Tryptose Blood Agar Base. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β-hemolytic streptococci.[2] Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[4]

Formula / Liter

Tryptose ..... 10 g
Beef Extract ..... 3 g
Sodium Chloride ..... 5 g
Agar ..... 15 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. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 33 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium, cooled to 45 - 50°C.

Quality Control Specifications

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

Prepared Appearance: Prepared medium without blood is yellow-beige, trace to light hazy. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Tryptose Blood Agar Base supplemented with 5% Sheep Blood and incubated aerobically at 35 ± 2°C and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Hemolysis
Escherichia coli ATCC® 25922	10 - 300	Fair to excellent	---
Streptococcus pyogenes ATCC® 19615	10 - 300	Fair to excellent	Beta hemolysis
Staphylococcus aureus ATCC® 25923	10 - 300	Fair to excellent	Beta hemolysis
Streptococcus pneumoniae ATCC® 6305	10 - 300	Fair to excellent	Alpha hemolysis

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

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, and stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[4]
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:[5]

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime-hemolysis (ά) 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 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 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. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human, and rabbit blood agar and alpha-hemolytic on sheep blood agar.[4]
2. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[4] For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Packaging

Tryptose Blood Agar Base	**Code No.**	**7282A**	**500 g**
		7282B	**2 kg**
		7282C	**10 kg**

References

1. **Casman, E. P.** 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. J. Bacteriol. **43:**33.
2. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci, and streptococci. Am. J. Clin. Pathol. **17:**281-289.
3. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
4. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
5. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. American Society for Microbiology, Washington, D.C

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.

NEOGEN CORPORATION
620 Lesher Place, Lansing MI 48912
517/372-9200 • 800/783-3212 • fax: 800/875-8563
neogen-info@neogen.com • www.neogen.com