

TRYPTIC SOY BLOOD AGAR BASE No. 2 (7654)

Intended Use

Tryptic Soy Blood Agar Base No. 2 is used with blood for the isolation and cultivation of a wide variety of microorganisms.

Product Summary and Explanation

Blood Agar Bases are typically supplemented with 5 - 10% sheep, rabbit, or horse blood, and 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.

Tryptic Soy Agar is based on the Soybean-Casein Digest formula specified by US Pharmacopeia.¹ Tryptic Soy Agar is a nutritious base, and a variety of supplements, including blood, are often added to enhance this medium. Tryptic Soy Blood Agar Base No. 2 was formulated to achieve good growth and improve hemolytic reactions of important pathogenic bacteria.

Blood Agar Base media are specified in standard methods for food testing.²

Principles of the Procedure

Tryptic Soy Blood Agar Base No. 2 is prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms. Tryptic Soy Blood Agar Base No. 2 contains two peptones, Enzymatic Digest of Casein, Type H and Enzymatic Digest of Soybean Meal, that provide nitrogen, carbon, and essential vitamins to stimulate organism growth. Along with essential nutritive properties, the peptones improve and enhance hemolysin production. 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.³ 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

Enzymatic Digest of Casein, Type H	15 g
Enzymatic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Agar	15 g

Final pH: 7.3 \pm 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 40 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 trace to slightly hazy and yellow-beige. With 5% sheep blood the medium is red and opaque.

Expected Cultural Response: Cultural response on Tryptic Soy Blood Agar Base No. 2 supplemented with 6% defibrinated sheep blood at 35°C after 18 - 48 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	good growth	slight beta hemolysis
<i>Listeria monocytogenes</i> ATCC® 7644	good growth	weak beta hemolysis
<i>Neisseria meningitidis</i> ATCC® 13090	good growth	non-hemolytic
<i>Staphylococcus aureus</i> ATCC® 25923	good growth	beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	good growth	alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	good growth	beta hemolysis

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

Test Procedure

1. 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.⁴
2. Incubate plates aerobically, anaerobically, or under conditions of increased CO₂ (5 - 10%).

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.
- 4) Alpha-prime-hemolysis (α') is a small zone of complete hemolysis, 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. The incubation environment can influence hemolytic reactions of beta-hemolytic streptococci.⁴
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood.⁴

Packaging

Tryptic Soy Blood Agar Base No. 2

Code No.	7654A	500	g
	7654B	2	kg
	7654C	10	kg

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Casman, E. P.** 1947. A noninfusion blood agar for neisseriae, pneumococci and streptococci. Am. J. Clin. 17:281-289.
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. Tenover (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. at TEL (800)783-3213 in the US/Canada or (410)780-5120 and (800)875-8563 in the US/Canada or (410)780-5470 for Technical Service involving dehydrated culture media preparation or performance.