

Tryptic Soy Blood Agar Base No.2
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NCM0098

Intended Use

Tryptic Soy Blood Agar Base No. 2 is used with blood for the isolation and cultivation of a wide variety of microorganisms in a laboratory setting. Tryptic Soy Blood Agar Base No. 2 is not intended for use in the diagnosis of disease or other conditions in humans.

Description

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. 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.

Typical Formulation

Enzymatic Digest of Casein	15.0 g/L
Enzymatic Digest of Soybean Meal	5.0 g/L
Sodium Chloride	5.0 g/L
Agar	15.0 g/L

Final pH: 7.3 ± 0.2 at 25°C

Formula is adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

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. Cooled to 45 - 50°C.
5. Prepare 5 - 10% blood agar by aseptically adding the appropriate volume of sterile defibrinated blood to melted sterile agar medium.

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 5-10% defibrinated sheep blood incubated aerobically at 35 ± 2°C and examined for growth after 18 - 48 hours.

Technical Specification Sheet



Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Hemolysis
<i>Escherichia coli</i> ATCC® 25922	10 - 300	Good to excellent	Slight Beta hemolysis
<i>Listeria monocytogenes</i> ATCC® 7644	10 - 300	Good to excellent	Weak Beta hemolysis
<i>Neisseria meningitidis</i> ATCC® 13090	10 - 300	Good to excellent	Non-hemolytic
<i>Staphylococcus aureus</i> ATCC® 25923	10 - 300	Good to excellent	Beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC® 6305	10 - 300	Good to excellent	Alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	10 - 300	Good to excellent	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.
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:

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.

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.
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

References

1. United States Pharmacopeia National Formulary 2018: USP 41 NF 35
2. Vanderzant, C., and D. F. Splittstoesser (eds.). 2015. Compendium of methods for the microbiological examination of foods, 4th 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.



Technical Specification Sheet



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