# 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 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.

## 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

Typtic 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 ± 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.

## 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 incubated aerobically at 35 ± 2°C and examined for growth after 18 - 48 hours.

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|---|---|---|---|
| | | Growth | Hemolysis |
| *Escherichia coli* ATCC® 25922 | 10 - 300 | Good to excellent | Slight Beta hemolysis |
| *Listeria monocytogenes* ATCC® 7644 | 10 - 300 | Good to excellent | Weak Beta hemolysis |
| *Neisseria menigitidis* ATCC® 13090 | 10 - 300 | Good to excellent | Non-hemolytic |
| *Staphylococcus aureus* ATCC® 25923 | 10 - 300 | Good to excellent | Beta hemolysis |
| *Streptococcus pneumoniae* ATCC® 6305 | 10 - 300 | Good to excellent | Alpha hemolysis |
| *Streptococcus pyogenes* 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.[4]
2. Incubate plates aerobically, anaerobically, or under conditions of increased $CO_2$ (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 (ά) 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.

## 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. 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.


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