# BLOOD AGAR BASE NO. 2 (7266)

## Intended Use

**Blood Agar Base NO. 2** is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms in a laboratory setting. 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 for use in isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, blood agar bases can be used as general purpose media.

In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis.[1] Blood Agar Base NO. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms. Blood Agar Base media are specified in standard method procedures for food testing.[2-4]

## Principles of the Procedure

Nitrogen, vitamin, and carbon sources are provided by Enzymatic Digest of Animal Tissue, Enzymatic Digest of Casein, Liver Digest, and Yeast Extract in Blood Agar Base NO. 2. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

## Formula / Liter

| | |
|---|---|
| Enzymatic Digest of Casein | 7.5 g |
| Enzymatic Digest of Animal Tissue | 7.5 g |
| Liver Digest | 2.5 g |
| Yeast Extract | 5 g |
| Sodium Chloride | 5 g |
| Agar | 12 g |

Final pH: 7.4 ± 0.2 at 25°C

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

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## Precautions

1. For Laboratory Use Only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

## Directions

1. Suspend 39.5 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 beige.

**Prepared Appearance:** Prepared medium without blood is light amber, and trace to slightly hazy. With 5% sheep blood, medium is red and opaque.

**Expected Cultural Response:** Cultural response on Blood Agar Based No. 2 with 5% sheep blood incubated at appropriate atmosphere and temperature and examined for growth after 18 - 72 hours incubation.

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|---|---|---|---|
| | | Growth | Reactions |
| *Escherichia coli* ATCC® 25922 | 10 - 300 | Good to excellent | - |
| *Neisseria meningitidis* ATCC® 13090 | 10 - 300 | Fair to good | - |
| *Staphylococcus aureus* ATCC® 25923 | 10 - 300 | Fair to good | Beta hemolysis |
| *Streptococcus pneumoniae* ATCC® 6305 | 10 - 300 | Fair | Alpha hemolysis |
| *Streptococcus pyogenes* ATCC® 19615 | 10 - 300 | Fair to good | Beta 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 oxygen-stable and oxygen-labile streptolysins.[5]
2. Incubate plates aerobically, anaerobically, or under conditions of increased $CO_2$ (5 - 10%) in accordance with established laboratory procedures.

## Results

Examine 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:[6]

1. Alpha hemolysis ($\alpha$) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis ($\beta$) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis ($\gamma$) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime hemolysis ($\alpha'$) is a small zone of complete hemolysis surrounded by an area of partial lysis.

## Storage

Store 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 expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance or has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

## Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. 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.[5]
3. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci.[5] For optimal performance, incubate blood agar base media under increased $CO_2$ (5 - 10%), in accordance with established laboratory procedures.

## Packaging

| | | | |
|---|---|---|---|
| **Blood Agar Base NO. 2** | **Code No.** | **7266A** | **500 g** |
| | | **7266B** | **2 kg** |
| | | **7266C** | **10 kg** |

## References


1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci. NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed., App. 3.08-3.09. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed., p. 1113. American Public Health Association, Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **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.
6. **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