

## Blood Agar Base (NCM0075)

### Intended Use

Blood Agar Base is used with blood for the isolation and cultivation of a wide variety of fastidious microorganisms. Blood Agar Base is not intended for use in the diagnosis of disease or other conditions in humans.

### Description

An inexpensive, general purpose agar base which, with the addition of 5-7% sterile blood, can be used to cultivate a wide range of microorganisms of indicative significance. Typical hemolysis patterns are obtained with this medium.

### Typical Formulation

Beef Extract	10.0 g/L
Balanced Peptone	10.0 g/L
Sodium Chloride	5.0 g/L
Agar	12.0 g/L

pH: 7.4 ± 0.2 at 25°C

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

### Precaution

Refer to SDS

### Preparation

1. Suspend 37 grams 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. Cool to 45-50°C and add 5-7% sterile defibrinated horse or sheep blood.

### Test Procedure

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

### Quality Control Specifications

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

**Prepared Appearance:** Prepared medium without blood (plain) is trace to slightly hazy and yellow-beige. With 5-7% horse blood, medium is red and opaque.



# Technical Specification Sheet



**Expected Cultural Response:** Cultural response on Blood Agar Base with 5-7% horse or sheep blood at 37 ± 1°C after 18 - 24 hours and 40-48 hours of incubation.

Microorganism	Approx. Inoculum (CFU)	Recovery	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	4Quad Streak	Growth	NA
<i>Escherichia coli</i> ATCC® 25922	4Quad Streak	Growth	NA
<i>Campylobacter jejuni</i> ATCC® 29428	50-200	≥ 70%	NA
<i>Campylobacter jejuni</i> ATCC® 33291	50-200	≥ 70%	NA
<i>Campylobacter coli</i> ATCC® 43478	50-200	≥ 70%	NA
<i>Pseudomonas aeruginosa</i> ATCC® 27853	4Quad Streak	Growth	NA
<i>Staphylococcus aureus</i> ATCC® 25923	4 Quad Streak	Growth	NA
<i>Streptococcus pneumoniae</i> ATCC® 6305	4Quad Streak	Growth	Alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC® 19615	50-200	≥ 70%	Beta hemolysis

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

## **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:

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.

## **Expiration**

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if 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.
2. Incubation atmosphere can influence hemolytic reactions of beta-hemolytic streptococci. For optimal performance, incubate blood agar base media under increased CO<sub>2</sub> (5 - 10%).

## **Storage**

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

## **References**

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