# PRODUCT SPECIFICATION SHEET

## Blood Agar Base w/ low pH (DM381)

**Intended Use**
Blood Agar Base w/ low pH (DM381) is an infusion medium, on addition of blood, is used for isolation and cultivation of fastidious microorganisms.

**Product Summary and Explanation**
Blood Agar Base is a highly nutritious medium generally used as a basal medium for preparing blood agar by supplementation with blood. 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. It can also be used as general-purpose media without the addition of blood. If the culture medium is to be used without addition of blood, the pH should be adjusted to 7.2 to 7.4, since most bacteria can grow better in a slightly alkaline medium.
In 1919, Brown experimented with blood agar formulations for the effects of colony formation and hemolysis. Lowering the pH in Blood Agar Base w/ LOW pH is used to enhance hemoytic reactions of streptococci. Blood Agar Base media are specified in standard method procedures for food testing.[1-3] The low pH of Blood Agar Base w/ low pH (pH 6.8) stabilizes the red blood corpuscles and favours the formation of clear zone of haemolysis.[4] Also it is advantageous for cultivation of *Streptococci* and *Pneumococci.* Blood Agar Base media can be used with added phenolphthalein phosphate[5] for the detection of phosphate producing staphylococci, with added salt and agar for assessment of surface contamination on equipment and pig carcass[6] and to determine salinity range of marine *Flavobacteria.*[7] It can also be used for preparation of *Salmonella typhi* antigens.[8]

**Principles of the Procedure**
Blood Agar Base w/ low pH contains beef extract and tryptose which provides carbon, nitrogen, amino acids and vitamins essential for growth of microorganisms. Sodium chloride helps in maintaining the osmotic equilibrium of the medium. Addition of blood makes the medium more nutritious by providing additional growth factors required by fastidious organisms. Haemolytic reactions are also visualized in presence of blood. However, haemolytic reactions depend on the animal blood used. Sheep blood gives best results for Group A *Streptococci* whereas; it fails to support growth of *Haemophilus haemolyticus* since sheep blood is deficient in pyridine nucleotides. However when horse blood is used *H.haemolyticus* colonies produce haemolysis and mimic *Streptococcus pyogenes.*

**Formula / Liter**

| Ingredients | Gms / Liter |
|---|---|
| Beef heart, infusion from (Beef extract) | 500.00 |
| Tryptose | 10.00 |
| Sodium chloride | 5.00 |
| Agar | 15.00 |
| Final pH: 6.8 ± 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.

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**Directions**

1. Suspend 40 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 50°C and aseptically add 5% v/v sterile defibrinated blood
5. Mix well and pour into sterile Petri plates.

**Quality Control Specifications**

| | |
|---|---|
| **Dehydrated Appearance** | Cream to yellow homogeneous free flowing powder |
| **Prepared Medium** | Basal medium: Light amber coloured clear to slightly opalescent gel<br>After addition of 5-7% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates |
| **Reaction of 4.0% Solution** | pH : 6.8 ± 0.2 at 25°C |
| **Gel Strength** | Firm, comparable with 1.5% Agar gel |

**Expected Cultural Response:** Cultural characteristics observed with added 5% w/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours

| Sr. No. | Organisms | Results to be achieved | | | | | |
|---|---|---|---|---|---|---|---|
| | | Inoculum (CFU) | Growth (w/o blood) | Recovery (w/o blood) | Growth (with blood) | Recovery (with blood) | Haemolysis |
| 1. | *Neisseria meningitides ATCC 13090* | 50 -100 | fair-good | 40-50% | good-luxuriant | >=70% | none |
| 2. | *Staphylococcus aureus ATCC 25923* | 50 -100 | good-luxuriant | 50-70% | good-luxuriant | >=70% | Beta |
| 3. | *Staphylococcus epidermidis ATCC 12228* | 50 -100 | good-luxuriant | 50-70% | good-luxuriant | >=70% | None |
| 4. | *Streptococcus pneumoniae ATCC 6303* | 50 -100 | fair-good | 40-50% | good-luxuriant | >=70% | Alpha |
| 5. | *Streptococcus pyogenes ATCC 19615* | 50 -100 | fair-good | 40-50% | good-luxuriant | >=70% | Beta |

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

**Test Procedure**

1. Process each specimen as appropriate, inoculate directly onto the surface of the medium. For isolation streak 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 $CO_2$ (5 - 10%) in accordance with established laboratory procedures for 18-48 hours.

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**Results**

Examine medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. Four types of hemolysis on blood agar media described as:

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

**Storage**

Store the sealed bottle containing the dehydrated medium at 10 - 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. 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 $CO_2$ (5 - 10%).
3. Consult appropriate texts for detailed information and recommended procedures.

**Packaging**

**Product Name : Blood Agar Base w/ low pH**
**Product Code : DM381**
**Available Pack sizes : 100gm / 500gm**

**References**


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4. Norton J. F., 1932, J. Lab. Clin. Med., 17:558-565.
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8. Schuber J. H., Edwards P. R. and Ramsere C. H., 1959, J. Bacteriol., 77:648.

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**Further Information**

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