# Tryptose Blood Agar Base with Yeast Extract

**M450**

Tryptose Blood Agar Base with Yeast Extract is recommended for the isolation of fastidious organisms and determining the haemolytic reactions.

## Composition**

| Ingredients | Gms / Litre |
|---|---|
| Tryptose | 10.000 |
| Beef extract | 3.000 |
| Yeast extract | 1.000 |
| Sodium chloride | 5.000 |
| Agar | 15.000 |
| Final pH ( at 25°C) | 7.3±0.2 |

**Formula adjusted, standardized to suit performance parameters

## Directions

Suspend 34 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For preparing Blood Agar cool the autoclaved medium to 45 - 50°C and aseptically add 5% v/v sterile defibrinated blood. Mix thoroughly, avoiding air bubbles and pour into sterile Petri plates.

## Principle And Interpretation

Tryptose Blood Agar Base w/ Yeast Extract is a tryptose based medium that can be used for the cultivation of fastidious organisms (1, 2), on supplementation with blood. This medium is devoid of dextrose and therefore useful in determining the haemolytic reactions.

Tryptose Blood Agar Base w/ Yeast Extract provides additional nutrients (yeast extract) to the fastidious organisms. Tryptose Blood Agar Base w/ Yeast Extract can be used as a general-purpose medium without supplementation of blood. This medium can be used to determine the heamolytic reactions of fastidious organisms. The four different types of haemolysis observed are as follows:

a) Alpha haemolysis: partial lysis of the erythrocytes surrounding a colony, causing a gray green or brownish discolouration in the media.

b) Beta haemolysis: complete lysis of the red blood cells surrounding a colony, causing a clearing of blood from the medium.

c) Gamma haemolysis: no haemolysis and consequently, no colour change of the medium surrounding a colony. Organisms showing no haemolysis are generally termed non-hemolytic rather than gamma haemolytic.

d) Alpha-prime or wide zone alpha: a small zone of intact erythrocytes immediately adjacent to the colony, with a zone of complete red cell haemolysis surrounding the zone of intact erythrocytes. This type of haemolysis may be confused with beta haemolysis (4).

Tryptose, beef extract and yeast extract provide nitrogenous and carbonaceous compounds, sulphur, vitamin B complex and trace elements essential for bacterial metabolism. Blood provides additional nutrients and serves as a base to study haemolytic reactions. This medium not only keeps the blood cells in a good state but also help in forming distinct haemolysis. Tryptose Blood Agar with Yeast Extract favours the good growth of *Neisseria meningitides* and *Streptococcus pneumoniae* . However, it can be used with or without blood supplementation. Perform biochemical test for further identification (3).

## Quality Control

**Appearance**

Cream to yellow homogeneous free flowing powder

**Gelling**

Firm, comparable with 1.5% Agar gel

**Colour and Clarity of prepared medium**
Basal medium : Yellow coloured clear to slightly opalescent gel forms in Petri plates. After addition of 5% v/v sterile defibrinated blood : Cherry red coloured opaque gel forms in Petri plates.

**Reaction**
Reaction of 3.4% w/v aqueous solution at 25°C. pH : 7.3±0.2

**pH**
7.10-7.50

**Cultural Response**
M450: Cultural characteristics observed with added 5% v/v sterile defibrinated blood, after an incubation at 35-37°C for 18-48 hours.

| Organism | Inoculum (CFU) | Growth w/o blood | Recovery w/o blood | Growth w/ blood | Recovery w/ blood | Haemolysis |
|---|---|---|---|---|---|---|
| *Neisseria meningitidis ATCC 13090* | 50-100 | luxuriant | >=70% | luxuriant | >=70% | none |
| *Staphylococcus aureus ATCC 25923* | 50-100 | luxuriant | >=70% | luxuriant | >=70% | beta |
| *Staphylococcus epidermidis ATCC 12228* | 50-100 | luxuriant | >=70% | luxuriant | >=70% | gamma |
| *Streptococcus pneumoniae ATCC 6303* | 50-100 | luxuriant | >=70% | luxuriant | >=70% | alpha |
| *Streptococcus pyogenes ATCC 19615* | 50-100 | luxuriant | >=70% | luxuriant | >=70% | beta |

## Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

## Reference
1. Casman E. P., 1942, J. Bacteriol., 43:33.
2. Casman E. P., 1947, Am. J. Clin. Pathol., 17: 281.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
4. Koneman E. W., Allen S. D., Janda W. M., Schreckenberger P. C. Winn W. C. Jr., 1992, Colour Atlas and Textbook of Diagnostic Microbiology, 4 th Ed., J. B. Lippinccott Company

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