

**Lysine Lactose Broth****M330**

Lysine Lactose Broth is used for determination of lysine decarboxylase activity of lactose non-fermenting members of *Enterobacteriaceae*, especially *Salmonellae*.

**Composition\*\*\***

Ingredients	Gms / Litre
Pancreatic digest of gelatin	5.000
Yeast extract	3.000
Dextrose	1.000
L-Lysine	5.000
Lactose	10.000
Bromocresol purple	0.020
Final pH ( at 25°C)	6.8±0.2

\*\*\*Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 24.02 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes in 5 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

**Principle And Interpretation**

The family *Enterobacteriaceae* consists of gram-negative facultatively anaerobic non-spore forming bacteria. These grow well on peptone meat extract media. However, several other factors have influenced the development of media for detection, isolation and enumeration of members of the *Enterobacteriaceae*. Decarboxylases are the enzymes that remove a molecule of CO<sub>2</sub> from an amino acid to form alkaline-reacting amines. Cadaverine is the amine degradation product of lysine. Many non-fermenters display only weak decarboxylase activity and many produce insufficient amines to convert the pH indicator system. This can be overcome by using only small quantities of substrates and heavy inoculum of pre-grown organisms in which a high concentration of enzymes has already accumulated. Overlaying the culture medium with 4 mm of petrolatum increases the sensitivity of detection. The initial conversion of the medium to a yellow colour, as acids accumulate from small amounts of glucose in the medium, is seen in case of the fermenters but not with the non-fermenters. The end point reactions are read comparing the strong alkaline purple colour reactions with the lighter bluish purple hue of the controls. Tubes should be incubated at 35°C for upto 5 days before interpreting the reactions as negative. Falkow (1) formulated Lysine Broth (It is also named as Falkow Lysine Broth) for detection of lysine decarboxylase by means of a colour reaction in enteric bacilli.

Pancreatic digest of gelatin and yeast extract provide nitrogenous and carbonaceous nutrients. Dextrose and lactose are the fermentable sugars. L-Lysine is the substrate that is decarboxylated due to decarboxylase enzyme activity. Bromocresol purple acts as the pH indicator. The enteric bacilli produce acid in an initial fermentation (lactose). Lactose non-fermenters produce acid from dextrose resulting in the formation of yellow colour. Subsequently L-Lysine is decarboxylated to form cadaverine resulting in an alkaline reaction and the broth reverts to purple colour.

**Quality Control****Appearance**

Cream to light green homogeneous free flowing powder

**Colour and Clarity of prepared medium**

Purple coloured clear solution without any precipitate

**Reaction**

Reaction of 2.40% w/v aqueous solution at 25°C. pH : 6.8±0.2

**Cultural Response**

M330: Cultural characteristics observed after an incubation at 35-37°C for 24 hours.

Organism	Inoculum (CFU)	Colour of medium	Lactose Fermentation	Lysine decarboxylation		
<i>Escherichia coli</i> ATCC 25922	50-100	yellow	positive reaction, yellow colour	negative reaction		

<i>Proteus vulgaris</i> ATCC 13315	50-100	bluish green	negative reaction	delayed positive reaction		
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	blue-purple	negative reaction	positive reaction, purple colour		
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	blue-purple	negative reaction	positive reaction, purple colour		
<i>Serratia marcescens</i> ATCC 8100	50-100	blue-purple	negative reaction	positive reaction, purple colour		

**Reference**

1. Falkow A., 1958, J. Clin. Pathol., 29:598

**Storage and Shelf Life**

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.