

## PHENOL RED AGAR BASE

For fermentation reactions of microorganisms

### Typical formula (g/l)

Peptocomplex	11.000
Sodium Chloride	5.000
Phenol Red	0.025
Agar	15.000

## PHENOL RED BROTH BASE

For fermentation reactions of microorganisms

### Typical formula (g/l)

Peptone	10.000
Beef Extract	3.000
Sodium Chloride	5.000
Phenol Red	0.018

### DIRECTIONS

Suspend 31g of Phenol Red Agar Base or 18g of Phenol Red Broth Base in 1000ml of cold distilled water. Heat to boiling, distribute the broth into fermentation tubes in amounts of 3-4 ml per tube, and the agar into Erlenmeyer flasks. Autoclave at 121°C 15 minutes. Cool to approximately 50°C and aseptically add a solution of appropriate carbohydrate sterilised by filtration at a final concentration of 1-2 % (w/v). Distribute the agar medium into sterile Petri dishes.

To reduce preparation time, paper discs containing carbohydrates (Bios Disc codes 191001-191020) can be added to the broth in the test tube, or onto the surface of the agar plate.

Final pH 7.4 ± 0.1

### DESCRIPTION

Phenol Red Agar Base and Phenol Red Broth Base, supplemented with the appropriate carbohydrates, are used to determine the fermentation reactions of microorganisms. Both media consist of a sugar free medium base, a pH indicator and a specific carbohydrate or related compound. Phenol Red Media produce acids when inoculated with bacteria that are capable of metabolising the substrate. Acid production causes a decrease in pH, which results in a colour shift in the medium.

### TECHNIQUE

#### **Solid medium method with paper discs (Bios Disc)**

Pour the sterilised medium into sterile plates (14mm diameter), allow to solidify and then inoculate the surface with a pure culture. For clearer and more rapid results, use the poured plate technique. Place the Carbohydrate Bios Disc on the surface of the inoculated agar, spacing them suitably so that they stick well to the medium (also inoculate a control plate without carbohydrates) and incubate at 37°C. A yellow zone around the discs indicates the production of acid.

#### **Liquid medium method with paper discs (Bios Disc)**

Aseptically introduce Bios Discs into the test tube of sterilised Phenol Red Broth Base, and inoculate with a loopful of pure culture. Also inoculate a control test tube without carbohydrate. Incubate at 37°C. Acid production is indicated by the colour of the culture changing to yellow.

With both media acid production occurs rapidly, and it is therefore, better to take frequent readings, the first after about 4 hours. After a positive reaction has been observed, discard the tube, because if the incubation period is prolonged, the reaction may be reversed with consequent indicator change to alkalinity.

### Storage

Dehydrated medium: 10-30°C

User prepared tubes: 1 month at 2-8°C

**REFERENCES**

- AOAC (1975) - Methods of Analysis. 12<sup>th</sup> edition.
- APHA (1963) - Diagnostic Procedures and Reagents, 4th edition..
- Edwards, P.R. & Ewing W.H. (1972) - Identification of *Enterobacteriaceae*. 3rd edition. Minneapolis: Burgess Publishing Company.
- International Committee on *Enterobacteriaceae* (1958) - Int. Bull. Bact. Nomencl. Tax., 8, 25.
- Sanders, A.C., Faber, J.E. & Cool., T.M. (1967) App. Microbiol., 5, 36.

**PACKAGING**

<b>4019052</b>	<b>Phenol Red Agar Base</b>	<b>500g (16.1 l)</b>
<b>4019102</b>	<b>Phenol Red Broth Base</b>	<b>500g (27.8 l)</b>