Asparagine Proline Broth, Granulated

GM1192

Asparagine Proline Broth, granulated is recommended for the cultivation of *Pseudomonas aeruginosa* from water samples using membrane filter technique.

Composition**

Ingredients	Gms / Litre
DL-Asparagine	2.000
L-Proline	1.000
Dipotassium phosphate, anhydrous	1.000
Magnesium sulphate	0.500
Potassium sulphate	10.000
Final pH	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 14.5 grams (for single strength medium) or 23.2 grams (for concentrated medium) in 1000 ml distilled water containing 25 ml or 40 ml ethanol respectively. Heat to boiling to dissolve the medium completely. Distribute as desired in screw-capped bottles. Close the caps so that the seal in the lid just touches the lip of the bottle. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Tighten the caps of the bottles immediately after removal from the autoclave to prevent loss of ethanol by evaporation.

It is not advisable to use polypropylene caps without seals. Alternatively, ethanol may be sterilized separately by filtration and then added aseptically to the sterile cooled medium.

Principle And Interpretation

Pseudomonas aeruginosa is one of the major contaminants of natural, fresh and recreational water, with the entry being contaminated by wastewater. *Ps. aeruginosa* is an opportunistic pathogen that can multiply in recreational waters in the presence of sufficient nutrients. It produces a water soluble, fluorescent pigment in media containing asparagine and ethanol. Asparagine Proline Broth is recommended for cultivation of *Ps. aeruginosa* by the membrane filter technique.in accordance with BIS standard (1).

Asparagine Proline Broth contains both the enantiomeric forms of Aspargine, which is readily utilized by *Pseudomonas* for their growth. Phosphate and sulphates provide the ions for the growth as well as buffers the medium to promote the growth of the organism.

When 1 ml of sample is to be analyzed, add 1 ml of sample to 4 ml of single strength medium (14.5 g/l). If larger portions of the sample (10 ml, 50 ml) are to be used, add the sample to an equal volume of the concentrated medium (23.2 g/l). Incubate at 37 ±1°C for 48 hours. Examine for growth and fluorescence. The growth is further sub cultured on Milk Agar w/ Cetrimide (M1273S).

Quality Control

Appearance
White to cream coloured granular medium
Colour and Clarity of prepared medium
Colourless clear solution, without any precipitate
Reaction
Reaction of 1.45% w/v aqueous solution at 25°C. pH : 7.2±0.2
pH
7.20-7.40
Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth
Cultural Response		
Escherichia coli ATCC 25922	50-100	none to poor
Pseudomonas aeruginosa ATCC 27853	50-100	luxuriant with greenish yellow pigment

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 label.

Reference

1. Bureau of Indian Standards (BIS), 2005, Draft IS 13428:2005

Revision : 00 / 2014