PKU Test Agar w/ Thienylalanine

M398

PKU Test Agar w/ Thienylalanine is recommended for estimation of phenylalanine in blood for detection of Phenylketonuria (PKU).

Composition**

Ingredients	Gms / Litre
L-Glutamic acid	0.500
DL-Alanine	0.500
Asparagine	0.500
Dextrose	10.000
Dipotassium phosphate	15.000
Monopotassium phosphate	5.000
Ammonium chloride	2.500
Ammonium nitrate	0.500
Sodium sulphate	0.500
Magnesium sulphate	0.050
Manganese chloride	0.005
Ferric chloride	0.005
Calcium chloride	0.0025
ß-2-Thienylalanine	0.0033
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 50.06 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. DO NOT AUTOCLAVE OR OVERHEAT. Cool to 50°C and add *Bacillus subtilis* spores. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Phenylketonuria is a congenital defect caused due to absence of phenylalanine hydroxylase. As a result of this, phenylalanine accumulates in the blood, which is excreted via urine hence it is called as phenylketonuria. Subsequently this deficiency may cause brain damage resulting in mental retardation. Guthrie and Tiekelmann (3) devised a modified inhibition assay for early detection of PKU using blood / urine samples of newborn infants having low levels of phenylalanine by determining the serum phenylalanine levels or the level of phenylpyruvic acid in urine.

The Guthrie test (1-4) was developed on the observation that *Bacillus subtilis* is normally inhibited in presence of b-2-thienylalanine but grows well when L-phenylalanine is added to the medium. Phenylalanine neutralizes the b-2-thienylalanine and allows bacteria to grow. The phenylalanine level can be read to determine the level of amino acid in blood. Other than phenylalanine, proline, phenylpyruvic acid or phenyllactic acid can be used. Small filter paper discs saturated with patients blood are placed on PKU Test Agar with b-2-thienylalanine inoculated with *Bacillus subtilis* . Control discs impregnated with different levels such as 2, 4, 6, 8,10,12 and 20 mg% of L-phenylalanine are also placed on the medium. After overnight incubation, zones of growth around the paper discs are observed and compared with zones around control discs. A response comparable to 4 mg% control disc is considered as presumptive positive. The results can be repeated using a duplicate test disc and a chemical or spectrofluorometric procedure (5, 6).

Quality Control

Appearance

Cream to greenish yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Light yellow coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 5% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M398: Cultural characteristics observed after an incubation at 35-37°C for 12-16 hours

Organism	Growth w/ 2% Phenylalanine	Growth w/ 4% Phenylalanine	Growth w/ 6% Phenylalanine	Growth w/ 8% Phenylalanine	Growth w/ 10% Phenylalanine	Growth w/ 12% Phenylalanine
Bacillus subtilis ATCC 6633	none-poor	luxuriant	luxuriant	luxuriant	luxuriant	luxuriant

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

Reference
1.Demain A. L., 1958, J. Bacteriol., 75:517.
2.Guthrie R., 1961, J. Am. Med. Assoc., 178:863.
3.Guthrie R. and Tiekelmann H., 1960, London Conference on the Scientific study of Mental Deficiency, London.
4.Guthrie R. and Susi A., 1963, Pediatrics, 32:338.
5.Ambrose J. A., Ingerson A., Gorrettson L. G., Chung L. W., 1967, Clin. Chem. Acta., 15:493.
6.Ambrose J. A., 1969, Clin. Chem., 15:15.

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