Technical Information

Toluidine Blue DNA Agar

Product Code: DM 1613I

Application: - Toluidine Blue DNA Agar is used for detection of thermostable deoxyribonuclease activity to establish speciation of *S.aureus* in contaminated foods. The composition and performance criteria of this medium are as per the specifications laid down in ISO 8870:2006(E) 83:2006(E).

Composition**

Ingredients	Gms / Litre
Deoxyribonucleic acid (DNA)	0.300
Calcium chloride	0.110
Sodium chloride	10.000
Toluidine blue	0.093
Tris (hydroxymethyl) amino methane	6.060
Agar	10.000
Final pH (at 25°C)	9.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Toluidine Blue DNA Agar (DM 1613) is recommended by APHA for detection of the thermostable deoxyribonuclease activity to establish the speciation of *S. aureus* in contaminated foods (1). Toluidine Blue DNA Agar (DM 1613I) is also recommended by ISO Committee (2) with a slight modification in concentration of calcium chloride and toluidine blue. The growth of *Staphylococcus aureus* in foods represents a potential public health hazard since many strains of *S.aureus* produce enterotoxins that cause food poisoning if ingested. Numerous outbreaks of staphylococcal intoxication are associated with cheese, stimulating numerous studies on the incidence and behaviour of staphylococci in milk and cheese. .

DNA In the medium enables the detection of DNase activity by getting depolymerized and forming a clear zone around the microbial growth. Inclusion of toluidine blue aids in detection of DNase activity by the production of a visible bright rose-pink coloured reaction due to its meta chromatic properties. Tris amino methane forms the buffering system. Sodium chloride and calcium chloride provide the ions. Sodium chloride & calcium chloride helps to maintain the osmotic equilibrium.

Methodology

Suspend 26.56 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boil to dissolve the medium completely and continue to boil for 1 to 2 minutes. Sterilization is not necessary. Dispense into sterile Petri plates.

Quality Control

Appearance
Light yellow to light grey homogeneous free flowing powder.

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity
Blue coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 2.65% w/v aqueous solution at 25°C. pH : 9.0±0.2

pH Range
8.80-9.20

Cultural Response
DM 1613I: 18 hours old BHI broth culture is heated in boiling water bath for 15 minutes and studied for thermonuclease activity. 5 mm cut well are cut in agar plates and is filled with 25-30µl of this culture and incubated at 35-37°C for 4 hrs (or it can also be incubated at 50°C for 2 hrs) and observed for results.

Organism	DNase activity
Staphylococcus aureus ATCC 12600	positive reaction, pink haloes extending 1mm beyond the well
Staphylococcus epidermidis ATCC 14990	negative reaction

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Downes F. P. and Ito K., (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
2. International Organization for Standardization ISO, 8870 :2006 (E), IDF, 83:2006 (E)

Disclaimer :

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