Toluidine Blue DNA Agar

M613

Toluidine Blue DNA Agar is used for detection of thermostable deoxyribonuclease activity.

Composition**

Ingredients	Gms / Litre
Deoxyribonucleic acid (DNA)	0.300
Calcium chloride	0.0055
Sodium chloride	10.000
Toluidine blue	0.083
Tris (hydroxymethyl) amino methane	6.100
Agar	10.000
Final pH (at 25°C)	9.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 26.48 grams in 1000 ml sterile distilled water. Heat to boiling to dissolve the medium completely and continue to boil for 1 or 2 minutes. Sterilization is not necessary. Dispense into sterile Petri plates.

Principle And Interpretation

Toluidine Blue DNA Agar is formulated as recommended by APHA (1) for the detection of thermostable deoxyribonuclease activity to establish speciation of *Staphylococcus aureus* in contaminated foods.

DNA in the medium enables the detection of DNase activity which depolymerizes the DNA resulting in the formation of 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 metachromatic properties. Tris amino methane forms the buffering system. Sodium chloride and calcium chloride provides the ions and also maintains osmotic equilibrium.

Quality Control

Appearance
Light yellow to light grey homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

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

Reaction
Reaction of 2.64% w/v aqueous solution at 25°C. pH : 9.0±0.2

pH
8.80-9.20

Cultural Response
M613: 18 hrs old BHI broth culture is heated in boiling water bath for 15 minutes and studied for thermonuclease activity. 5 mm cut wells 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

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

Reference

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.

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