

TM 2374 – TOLUIDINE BLUE DNA AGAR (ISO 8870:2006(E) 83:2006(E)

INTENDED USE

For detection of thermostable deoxyribonuclease activity to establish speciation of *S.aureus* in contaminated foods.

PRODUCT SUMMARY AND EXPLANATION

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. Toluidine Blue DNA Agar is recommended by APHA for detection of the thermostable deoxyribonuclease activity to establish the speciation of S. aureus in contaminated foods. Toluidine Blue DNA Agar is also recommended by ISO Committee with a slight modification in concentration of calcium chloride and toluidine blue.

COMPOSITION

Ingredients	Gms / Ltr	
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	

PRINCIPLE

The medium consists of DNA which 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 metachromatic properties. Tris amino methane forms the buffering system. Sodium chloride and calcium chloride provide the ions and also maintains osmotic equilibrium.

INSTRUCTION FOR USE

- Dissolve 26.56 grams in 1000 ml distilled water.
- Heat to boiling 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 SPECIFICATIONS

Appearance of Powder : Light yellow to light grey homogeneous free flowing powder. Appearance of prepared medium : Blue coloured clear to slightly opalescent gel forms in Petri plates.

pH (at 25°C) $: 9.0 \pm 0.2$

INTERPRETATION

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 and observed for results.













Microorganism	ATCC	Inoculum (CFU/ml)	DNase activity	Incubation temperature	Incubation Period
Staphylococcus aureus	12600	50-100	Positive reaction, pink halos extending 1mm beyond	35-37°C	4 Hours or 50°C for 2 Hours
Staphylococcus epidermidis	14990	50-100	Negative reaction	35-37°C	4 Hours or 50°C for 2 Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

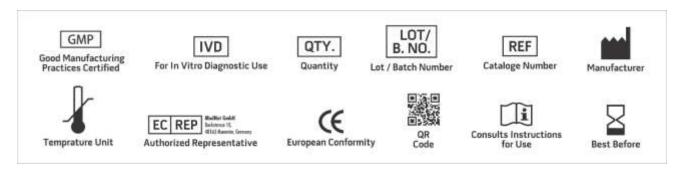
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- 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).



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*For Lab Use Only
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