TM 2375 – TOLUIDINE BLUE DNA AGAR, MODIFIED

INTENDED USE

For detection of thermostable deoxyribonuclease activity and establish speciation of *S. Aureus* in contaminated foods in accordance with FDA BAM, 1998.

PRODUCT SUMMARY AND EXPLANATION

Toluidine Blue DNA Agar, Modified is recommended for detection of thermostable deoxyribonuclease activity and establish speciation of S. aureus in contaminated foods in accordance with FDA BAM, 1998. *Staphylococcus* is Gram-positive bacteria which includes several species that can cause a wide variety of infections in humans and other animals through infection or the production of toxins. Staphylococcal intoxication worldwide stands out as one of the main food-borne diseases, with a frequency in incidents of microbiological origin second only to salmonellosis. Foods that lack hygienic conditions and that are improperly stored can aid in the Staphylococcal growth and toxin production. Since the infection is caused due to toxin, Staphylococcal food poisoning is also called as 'food intoxication'. Toluidine Blue DNA Agar, Modified is recommended for detection of thermostable deoxyribonuclease activity and establish speciation of S. aureus in contaminated foods in accordance with FDA BAM, 1998 with a slight modification in concentration of calcium chloride and toluidine blue. 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 metachromatic properties.

Total plate count of the suspected sample is carried out using Baird Parker Agar. Speciation of the organism can be confirmed using Toluidine Blue DNA Agar, Modified. Like the coagulase test, this is a highly specific test for the confirmation of the organism. The test is carried out in petriplates or microslides containing the media, prepared by spreading appropriate amount (3ml if slide) of the Toludine Blue DNA Agar, Modified medium. 10 to 12 wells of 1mm diameter are prepared in the plates/slides after solidification. Add 0.01 ml of the broth culture into these wells after heating in a boiling water bath for 15min. Incubate (in a moist chamber) for 4 hrs at 35°C. Development of bright pink halo extending at least 1 mm from periphery of well indicates a positive reaction. Tris amino methane forms the buffering system. Sodium chloride and calcium chloride provide the ions and also maintains osmotic equilibrium.

COMPOSITION

Ingredients	Gms / Ltr
Deoxyriboneuclic acid (DNA)	0.300
Calcium chloride (anhydrous)	0.0011
Sodium chloride	10.000
Toludine blue	0.083
Tris (hydroxy methyl) amino methane	6.100
Agar	10.000

PRINCIPLE

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.

INSTRUCTION FOR USE

- Dissolve 26.48 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±0.2

INTERPRETATION

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 and observed for results.

Microorganism	ATCC	Inoculum (CFU/ml)	DNase activity	Incubation Temperature	Incubation Period
Staphylococcus aureus	12600	50-100	Positive reaction, pink haloes extending 1mm beyond the well	35-37°C	4 Hours
Staphylococcus epidermidis	14990	50-100	Negative reaction	35-37°C	4Hours

PACKAGING:

In pack size of 100 gm bottles.

STORAGE

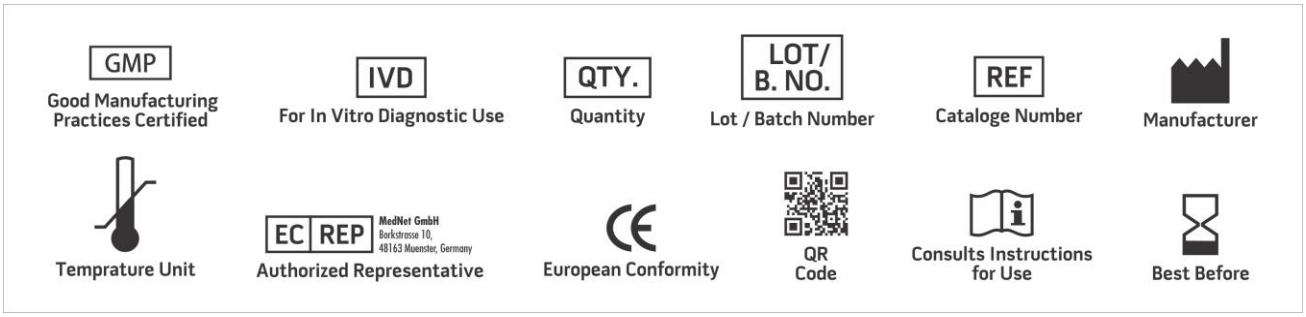
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided 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.Bautista, L., Gaya, P., Medina, M. and Nuñez, M. 1988. Applied and Environmental Microbiology, 54(2): 566-569.
2.Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A. and Yolken, R. H. 2003. Manual of Clinical Microbiology. 8 ed. Washington, D.C: ASM.
3.FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.



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

***For Lab Use Only**

Revision: 08 Nov., 2019