

TM 2259 – NUTRIENT AGAR W/TRYPAN BLUE

INTENDED USE

Recommended for detection and enumeration of aerobic endospore formers from water samples by membrane filtration.

PRODUCT SUMMARY AND EXPLANATION

Nutrient Agar with Trypan blue is recommended for the detection of aerobic endospores from water samples by APHA. Endospores are formed by certain genera of bacteria, commonly found in soil and water samples. Under favorable conditions of growth medium, endospores are readily converted to vegetative cells. The water samples under test is exposed to heat treatment wherein vegetative cells are destroyed and aerobic spores remain unaffected. The water sample is then filtered through membrane filter and placed on Agar plate. Incubate at $35 \pm 0.5^\circ\text{C}$ for 24 ± 2 hours. Further incubation upto 5-7 days for intracellular formation of endospores. Count the number colonies as aerobic endospores. The colonies should be catalase positive.

COMPOSITION

Ingredients	Gms / Ltr
Peptone	5.000
Beef extract	3.000
Agar	15.000
Trypan blue	0.015

PRINCIPLE

The medium consists of Peptone and Beef extract that provide the necessary nitrogen compounds, carbon, long chain amino acids, vitamins and also some trace ingredients necessary for the growth of bacteria. Trypan blue is added to impart a coloured background to the membrane. The aerobic endospores can be easily visualized.

INSTRUCTION FOR USE

- Dissolve 23.02 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to $45-50^\circ\text{C}$.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Light yellow to blue homogeneous free flowing powder.
Appearance of prepared medium	: Light blue coloured clear to slightly opalescent gel forms in Petri plates.
pH (at 25°C)	: 6.8 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
<i>Bacillus subtilis</i> <i>subsp. spizizenii</i>	6633	50-100	Good-luxuriant	50-70%	35 ± 0.5°C	22-26 Hours

PACKAGING:

In pack size of 100 gm and 500 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. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

 GMP Good Manufacturing Practices Certified	 Best Before	 QTY. Quantity	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 LOT/ B. NO. Lot / Batch Number	 Consults Instructions for Use	 QR Code	

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

***For Lab Use Only**
Revision: 08 Nov., 2019