



## Nutrient Agar w/Trypan blue

M2051

### Intended use

Nutrient Agar w/Trypan blue is recommended for detection and enumeration of aerobic endospore formers from water samples by membrane filtration.

### Composition\*\*

Ingredients	Gms / Litre
Peptone	5.000
HM peptone B#	3.000
Trypan blue	0.015
Agar	15.000
Final pH ( at 25°C)	6.8±0.2

\*\*Formula adjusted, standardized to suit performance parameters

# - Equivalent to Beef extract

### Directions

Suspend 23.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

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. Nutrient Agar with Trypan blue is recommended for the detection of aerobic endospores from water samples by APHA (1). 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±0.5°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.

Peptone and HM peptone B 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.

### Type of specimen

Water samples.

### Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (1). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations :

1. This medium is recommended for enumeration of aerobic endospore formers.
2. Further biochemical tests must be carried out for confirmation.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Light yellow to blue homogeneous free flowing powder

### Gelling

Firm, comparable with 1.5% Agar gel

### Colour and Clarity of prepared medium

Light blue coloured clear to slightly opalescent gel forms in Petri plates

### Reaction

Reaction of 2.3% w/v aqueous solution at 25°C. pH : 6.8±0.2

### pH

6.60 - 7.00

### Cultural Response

Cultural characteristics observed after an incubation at 35±0.5°C for 22-26 hours.

Organism	Inoculum (CFU)	Growth
<i>Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)</i>	50-100	good-luxuriant

Key : (\*) corresponding WDCM numbers

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

## Reference

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 2<sup>nd</sup> 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.

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### Disclaimer :

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