

# TMP 009 – R-2A AGAR PLATE

#### **INTENDED USE**

For heterotrophic plate count of treated potable water, using longer incubation period.

#### PRODUCT SUMMARY AND EXPLANATION

The heterotrophic plate count (HPC), formerly known as the standard plate count is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment, in distribution systems or in swimming pools. R-2A Agar is recommended by APHA for estimating the heterotrophic plate count by the pour plate, spread plate or membrane filter procedure. R-2A Agar is formulated as per Reasoner and Geldreich. Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former. Therefore the use of a low nutrient medium like R-2A Agar incubated for longer incubation periods allows these stressed organisms to grow well.

Many bacteria from natural waters which contain limited nutrients at ambient temperature, grow best on the media with less nutrient levels. They grow better at the temperatures below the routine laboratory incubation temperatures of 35 to 37°C.

#### **COMPOSITION**

Ingredients	Gms / Ltr	
Agar	15.000	
Sodium pyruvate	0.300	
Yeast extract	0.500	
Proteose peptone	0.500	
Dextrose	0.500	
Starch Soluble	0.500	
Dipotassium hydrogen phosphate	0.300	
Magnesium sulphate	0.024	
Casein Acid Hydrolysate	0.500	

#### **PRINCIPLE**

Casein acid hydrolysate, proteose peptone and yeast extract provide nitrogen, carbon compounds, vitamins, amino acids and minerals. Dextrose/ glucose serves as an energy source. Magnesium sulphate is a source of divalent cations and sulphate. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Dipotassium hydrogen phosphate is used to balance the pH of the medium. The number of colonies on a plate are reported as CFU (Colony Forming Units) per volume of sample.

## **INSTRUCTION FOR USE**

Either streak, inoculate or surface spread the test inoculum aseptically on the plate.

## **QUALITY CONTROL SPECIFICATIONS**

**Appearance** Light yellow colored medium. 25ml of medium in 90mm plates. **Quantity of Medium** 

pH (at 25°C)  $7.2 \pm 0.2$ 

**Sterility Check** Passes release criteria











#### INTERPRETATION

Cultural response was observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Good- Luxuriant	>=50%	35-37°C	24-72 hours
Escherichia coli	8739	50-100	Good- Luxuriant	>=50%	35-37°C	24-72 hours
Bacillus subtilis subsp. spizizenni	6633	50-100	Good- Luxuriant	>=50%	35-37°C	24-72 hours
Enterococcus faecalis	29212	50-100	Good- Luxuriant	>=50%	35-37°C	24-72 hours
Staphylococcus aureus subsp. aureus	25923	50-100	Good- Luxuriant	>=50%	35-37°C	24-72 hours
Aspergillus brasilienses	16404	10-100	Good- Luxuriant	>=50%	35-37°	24-72 hours
Candida albicans	10231	50-100	Good- Luxuriant	>=50%	35-37°	24-72 hours
Salmonella enteritidis	13076	50-100	Good- Luxuriant	>=50%	35-37°	24-72 hours
Pseudomonas aeruginosa	9027	50-100	Good- Luxuriant	>=50%	35-37°	24-72 hours
Salmonella typhi	6539	50-100	Good- Luxuriant	>=50%	35-37°	24-72 hours

## **PACKAGING:**

Doubled layered packing containing 5 No. of plates with one silica gel desiccant bag packed inside it.

## **STORAGE**

On receipt, store the plates at 15–30 °C. Avoid freezing and overheating. Do not open until ready to use. Prepared plates stored in their original sleeve wrapping until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

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

## **DISPOSAL**

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

### **REFERENCES**

- 1. Clesceri L. S., Greenberg A. E. and Eaton A. D., (Ed.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., American Public Health Association, Washington, D.C.
- 2. Downes F. P. and Ito K., (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., American Public Health Association, Washington, D.C.
- 3. Reasoner D. J. and Geldreich E. E., 1985, Appl. Environ. Microbiol., 49:1.
- 4.Collins V. J. and Willoughby J. G., 1962, Arch. Microbiol., 43:294.











# **PRODUCT DATA SHEET**











GMP Certification of Good Manufacturing Practices









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

\*For Lab Use Only

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