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R- 2A AGAR

Used for heterotrophic plate count of water samples using longer incubation periods.

REF: BS.1/DB01.100.0100	100 Gram	REF: BS.1/DB01.250.0250	250 Gram
REF: BS.1/DB01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

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 (1, 2) 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 (3). Stressed or injured organisms during water treatment are unable to grow on high nutrient media, since the faster growing organisms outgrow the former (4). 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 (4). Acicase, proteose peptone and yeast extract provide nitrogen, carbon compounds, vitamins, amino acids and minerals. Dextrose/ glucose serves as an energy source. Soluble starch aids in the recovery of injured organisms by absorbing toxic metabolic byproducts while sodium pyruvate increases the recovery of stressed cells. Magnesium sulphate is a source of divalent cations and sulphate. 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.

MEDIA COMPOSITION

Item	Formula per liter of medium g/l
- Casein Acid Hydrolysate	0.5
- Peptone	0.5
- YEAST extract	0.5
- Dextrose	0.5
- Starch soluble	0.5
- Dipotassium hydrogen phosphate	0.3
- Magnesium sulphate	0.024
- Sodium pyruvate	0.3
- Agar	15

pH 7.2 ± 0.2 @ 25°C

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental Contamination.
S61: avoid release in environment.
For further information, refer to the R – 2A AGAR material safety data sheet.

STORAGE AND STABILITY

BioScien R – 2A AGAR dehydrated media are stable until expiration date stated on label when properly stored 10-30°C. The prepared medium should be stored at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a 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.

MEDIA PREPARATION

Suspend 18.12 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes or as per validated cycle. DO NOT OVERHEAT. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Deterioration

The color of BioScien R– 2A AGAR is Cream to yellow homogeneous free flowing powder. Colour and Clarity of prepared medium IS Light yellow coloured clear to slightly opalescent gel forms in Petri plates in Petri plates If there are any physical changes, discard the medium The hydrated medium is Amber coloured clear solution without any precipitate, media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), and contaminations.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile cups
- Sterile tubes
- Sterile loops
- Incubator

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed *by using standard ATCC cultures after an incubation at 30-35°C for 24-72 hours. (*-In case of water samples from fields it is suggested to incubate further for upto 7 days to ascertain the absence of organisms)

Test Organisms	Growth
Candida albicans ATCC 10231	Good -Luxuriant
Escherichia coli ATCC 25922	Good -Luxuriant
Salmonella Enteritidis *ATCC 13076*	Good -Luxuriant
Pseudomonas paraeruginosa ATCC 9027	Good -Luxuriant
Staphylococcus aureus subsp. *Aureus ATCC 6538*	Good -Luxuriant
Enterococcus faecalis ATCC 29212	Good -Luxuriant

SYMBOLS IN PRODUCT LABELLING	
IVD For in-vitro diagnostic use	Number of <n> test in the pack
LOT Batch Code/Lot number	Caution
REF Catalogue Number	Do not use if package is damaged
Temperature Limitation	Consult Instruction for use
Expiration Date	
Manufactured by	

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact ***BioScien*** technical support.

REFERENCES

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