

Technical Information

Brilliant Green Agar with Phosphates

Product Code: DM 1971

Application: - Brilliant Green Agar with Phosphates is used for selective isolation and identification of Salmonellae from mixed flora while inhibiting *Escherichia coli*, *Proteus* and *Pseudomonas specie*.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Beef extract	5.000
Yeast extract	3.000
Lactose	10.000
Sucrose	10.000
Disodium phosphate	1.000
Monosodium phosphate	0.600
Phenol red	0.090
Brilliant green	0.0047
Agar	12.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Salmonella species cause many types of infections, ranging from mild self-limiting gastroenteritis to life threatening typhoid fever. The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting for less than 2 days and diarrhea up to 7 days ⁽¹⁾.

Brilliant Green Agar Base w/phosphates is formulated as per the recommendation of Rijks Institute Voorde Volksgezondheid (National Institute for Public Health), Utrecht ^(2, 3). It is also recommended by the ISO Committee ^(4, 5, 6), because of its ability to recover smaller numbers of *Salmonella* species, and at the same time inhibiting the growth of *Escherichia coli*, *Proteus species* and *Pseudomonas species* ⁽⁷⁾.

The medium contains peptic digest of animal tissue, beef extract and yeast extract as sources of carbon, nitrogen, vitamins, amino acids and essential nutrients. The two sugars namely lactose and sucrose serve as energy sources. Fermentation of lactose and / or sucrose in the medium results in the formation of acidic pH which is detected by phenol red indicator. Phosphates (DM1971) buffer the medium. Brilliant green helps to inhibit the growth contaminating microflora. The medium can further supplemented with sulphacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit the growth of contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species ⁽⁸⁾.

Brilliant Green Agar w/Phosphates being highly selective is recommended to be used along with a less inhibitory medium to improve the chances of recovery of other pathogens. Often cultures are enriched in Selenite Cystine Broth (DM1025) or Tetrathionate Broth (DM1032). These enriched cultures are then isolated simultaneously on Brilliant Green Agar Base (DM1016/DM1971), SS Agar (DM1108), Bismuth Sulphite Agar (DM1027) and MacConkey Agar (DM1081).

Methodology

Suspend 25.84 grams of powder media in 500 ml distilled water. Shake well & heat with occasional agitation and bring just to the boil to dissolve the medium completely. DO NOT AUTOCLAVE. For more selectivity and maximum recovery aseptically add the rehydrated contents of 1 vial of Sulpha Supplement (MS2068). Mix well before pouring into sterile Petri plates

Quality Control

Physical Appearance

Light yellow to pink homogeneous free flowing powder

Gelling: Firm, comparable with 1.2% Agar gel.

Colour and Clarity of Prepared Medium

Greenish brown coloured clear to slightly opalescent gel forms in petri plates.

Reaction

Reaction of 5.2% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH Range 6.70-7.10

Cultural Response/Characteristics

DM 1971: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Inoculum (CFU)	Recovery	Colour of colony	Colour of colony
<i>Escherichia coli</i> ATCC 25922	>=10 ³	inhibited	0%	
<i>Proteus vulgaris</i> ATCC 13315	50-100	none-poor	<=10%	Red
<i>Pseudomonas aeruginosa</i> ATCC 10145	50-100	none-poor	<=10%	Red
<i>Salmonella Enteritidis</i> ATCC 13076	50-100	Luxuriant	<=50%	bright red
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	<=50%	bright red

Storage and Shelf Life

Dried media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.

Prepared Media: 2-8⁰ in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Edel W. and Kampelmacher E. H., 1969, Bull. W.H.O., 41:297.
3. Edel W. and Kampelmacher E. H., 1969, Bull. W.H.O., 39:487.
4. Anon, 1975, International Organization for Standardization, Meat and Meat products Ref. Method, ISO: 3565.
5. Anon, 1981, International Organization for Standardization, Microbiology Ref. Methods, ISO: 6579.
6. Anon, 1985, International Organization for Standardization, Milk and Milk Products; Ref. Method, ISO: 6785.
7. Read R. B. and Reyes A. L., 1968, Appl. Microbiol., 16:746.
8. Watson U. C. and Walker A. P., 1978, J. Appl. Bacteriol. 45:195.

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