

## Brilliant Green HiCynth™ Agar Base, Modified

MCD016

Brilliant Green HiCynth™ Agar Base, Modified is used for selective isolation of Salmonellae other than *Salmonella* Typhi from faeces, food and dairy products.

### Composition\*\*

Ingredients	Gms / Litre
HiCynth™ Peptone No.5*	3.000
HiCynth™ Peptone No.4*	10.000
Lactose	10.000
Sucrose	10.000
Sodium chloride	5.000
Phenol red	0.080
Brilliant green	0.0125
Agar	20.000
Final pH ( at 25°C)	6.9±0.2

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

\*Chemically defined peptones.

### Directions

Suspend 29.05 grams in 500 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. AVOID OVERHEATING. Cool to 45-50°C. For more selectivity, aseptically add rehydrated contents of 1 vial of Sulpha Supplement (FD068). Mix well and pour into sterile Petri plates.

### Principle And Interpretation

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

Brilliant Green HiCynth™ Agar Base, Modified, as a primary plating medium for isolation of *Salmonella* species was first described by Kristensen et. al. (1) and further modified by Kauffmann (2). Brilliant Green Agar is also recommended by APHA (3,4) FDA (5) and described in EP, BP and IP (6,7,8).

This medium contains brilliant green, which inhibits growth of majority of Gram-negative and Gram-positive bacteria. *Salmonella* Typhi, *Shigella* species, *Escherichia coli*, *Pseudomonas* species, *Staphylococcus aureus* are mostly inhibited. Clinical specimens can be directly plated on this medium. However, being highly selective, it is recommended that this medium should be used along with a less inhibitory medium to increase the chances of recovery.

The medium contains HiCynth™ Peptone No.4 and HiCynth™ Peptone No.5 as sources of carbon, nitrogen, long chain amino acids, vitamins 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. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulphacetamide (1g/l) and sodium mandelate (0.25g/l) to inhibit contaminating microorganisms when the sample is suspected to contain large number of competing organisms along with *Salmonella* species.

Non-lactose fermenting bacteria develop white to pinkish red colonies within 18-24 hours of incubation. *Salmonella* Typhi and *Shigella* species may not grow on this medium. Moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.

### Quality Control

#### Appearance

Please refer disclaimer Overleaf.

Light yellow to light pink homogeneous free flowing powder

### Gelling

Firm, comparable with 2.0% agar gel.

### Colour and Clarity of prepared medium

Greenish brown clear to slightly opalescent gel forms in Petri plates

### Reaction

Reaction of 5.81% w/v aqueous solution at 25°C. pH : 6.9±0.2

### pH

6.70-7.10

### Cultural Response

Cultural response was carried out after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

### Cultural Response

Organism	Inoculum (CFU)	Growth	Lot value (CFU)	Recovery	Colour of Colony
<b>Cultural Response</b>					
<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	good-luxuriant	25 -100	≥50 %	pinkish white
<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	25 -100	≥50 %	pinkish white
<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	25 -100	≥50 %	pinkish white
<i>Salmonella Typhi</i> ATCC 6539	50 -100	fair-good	15 -40	30 -40 %	reddish pink
<i>Escherichia coli</i> ATCC 25922	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Escherichia coli</i> ATCC 8739	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Escherichia coli</i> NCTC 9002	50 -100	none-poor	0 -10	0 -10 %	yellowish green
<i>Staphylococcus aureus</i> ATCC 25923	≥10 <sup>3</sup>	inhibited	0	0%	
<i>Staphylococcus aureus</i> ATCC 6538	≥10 <sup>3</sup>	inhibited	0	0%	

### Storage and Shelf Life

Store below 30°C in a tightly closed container and the prepared medium between 2 - 8°C. Use before expiry date on the label.

### Reference

- Kristensen M., Lester V, and Jurgens A., 1925, Brit.J.Exp.Pathol.,6:291.
- Kauffman F., 1935, Seit F. Hyg, 177:26.
- Downes F. P. and Ito K. (Ed), 2001, Compendium of Methods for Microbiological Examination of Foods, 4th Ed. APHA, Washington D.C.
- Standard Methods for the Microbiological Examination of Dairy Products, 1995, 19th Ed, APHA, Washington, D.C.
- Bacteriological Analytical Manual, 5th Ed, 1978, AOAC, Washington D.C.
- The European Pharmacopoeia, 2014, Council or Europe, Strasbourg.
- The British Pharmacopoeia, 2016 vol. II, London.
- Indian Pharmacopoeia, 2014, Ministry of Health and Family Welfare, Govt., of India.

Revision : 00 / 2015

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