

## Brilliant Green HiVeg<sup>®</sup> Agar Base, Modified

MV016

### Intended Use:

Recommended for selective isolation of *Salmonellae* other than *Salmonella* Typhi from food and dairy products and other materials.

### Composition\*\*

Ingredients	g / L
HiVeg <sup>®</sup> peptone No. 3	10.000
Yeast extract	3.000
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

### Directions

Suspend 58.09 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. **AVOID OVERHEATING.** For more selectivity, aseptically add rehydrated contents of 2 vials 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 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). Brilliant Green HiVeg<sup>®</sup> Agar Base, Modified is same as Brilliant Green Agar Base, Modified except that the animal peptones are completely replaced by vegetable peptones to avoid BSE/TSE risks associated with animal peptones.

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. 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. Often cultures enriched in Selenite (MV025) or Tetrathionate HiVeg<sup>®</sup> Broth (MV032) are plated on Brilliant Green HiVeg<sup>®</sup> Agar Base, Modified as well as Bismuth Sulphite HiVeg<sup>®</sup> Agar (MV027), SS HiVeg<sup>®</sup> Agar (MV108) and MacConkey HiVeg<sup>®</sup> Agar (MV081). The medium contains HiVeg<sup>®</sup> peptone No. 3 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. Sodium chloride maintains the osmotic equilibrium. Brilliant green helps to inhibit the contaminating microflora. The medium can further supplemented with sulphaacetamide (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.

### Type of specimen

Foodstuffs & dairy samples; Water samples; Pharmaceutical samples.

### Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,4).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (5)

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (6,7,8). 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. Though this medium is selective for *Salmonella* other species of *Enterobacteriaceae* may grow.
2. *Salmonella* Typhi and *Shigella* species may not grow on this medium.
3. Moreover *Proteus*, *Pseudomonas* and *Citrobacter* species may mimic enteric pathogens by producing small red colonies.
4. Further confirmation has to be carried out on presumptive *Salmonella* isolates.

## 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 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 Petriplates

### Reaction

Reaction of 5.8% 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.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of Colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	none-poor	0 -10 %	yellowish green
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	none-poor	0 -10 %	yellowish green
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	≥10 <sup>4</sup>	inhibited	0%	
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 6538 (00032*)	≥10 <sup>4</sup>	inhibited	0%	
<i>Salmonella</i> Typhi ATCC 6539	50 -100	fair-good	30 -40 %	reddish pink
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	good-luxuriant	≥50 %	pinkish white
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	≥50 %	pinkish white
<i>Salmonella</i> Abony NCTC 6017 (00029*)	50-100	good-luxuriant	≥50 %	pinkish white

Key : \*Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium 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 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 (12,13)

## Reference

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