BG Sulfag Aga
SBG Sulfag Enrichment

**Intended Use**
BG Sulfag Agar is used for isolating Salmonella.
SBG Sulfag Enrichment is used for enriching Salmonella prior to isolation procedures.

**Summary and Explanation**
Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of Salmonella in domesticated animals. Infection with non-typhi Salmonella often causes mild, self-limiting illness. The illness results from consumption of raw, undercooked or improperly processed foods contaminated with Salmonella. Many of these cases of Salmonella-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for Salmonella before their distribution for human consumption, but in many instances, contaminated food samples elude detection.

BG (Brilliant Green) Sulfag Agar is a highly selective medium. Osborne and Stokes added 0.1% sodium sulfapyridine to Brilliant Green Agar to enhance the selective properties of this medium for Salmonella. This formula is recommended as a selective isolation medium for Salmonella following enrichment. It is also recommended for direct inoculation with primary specimens for Salmonella isolation.

For food testing, BG Sulfag Agar has been used for detection of Salmonella in low and high moisture foods. It has also been used for detecting Salmonella in feeds and feed ingredients. This medium is recommended when testing foods for Salmonella following USDA guidelines.

SBG (Selenite Brilliant Green) Sulfag Enrichment is prepared according to the formula described by Stokes and Osborne. The researchers found that whole egg and egg yolk reduced the selective properties of selenite brilliant green enrichment. They also found that the addition of sulfapyridine (SBG Sulfag Enrichment) restored these selective properties.

SBG Sulfag Enrichment is a selective enrichment for the isolation of Salmonella species, especially from egg products. The shell and the contents of the egg at the time of oviposition are generally sterile or harbor very few microorganisms. Contamination of the shell occurs afterwards from nesting material, floor litter and avian fecal matter.

**Principles of the Procedure**
In BG Sulfag Agar, peptone and yeast extract provide nitrogen, vitamins and minerals. Lactose and sucrose are the sources of carbohydrates in the medium. Brilliant green and sodium pyridine are complementary in inhibiting gram-positive bacteria and most gram-negative bacilli other than Salmonella spp. Phenol red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Peptone provides the nitrogen, minerals and amino acids in SBG Sulfag Enrichment. Yeast extract is the vitamin source. D-Mannitol is the carbon source to stimulate organism growth. The phosphates act as buffers in the enrichment. Sodium taurocholate, sodium selenite and brilliant green are the selective agents. The selective agents are used to inhibit gram-positive organisms and enteric bacteria other than Salmonella. Sodium sulfapyridine is added to increase selectivity.

**Formulae**

**Difco™ BG Sulfag Agar**

<table>
<thead>
<tr>
<th>Approximate Formula* Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast Extract</td>
</tr>
<tr>
<td>Protease Peptone No. 3</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Saccharose</td>
</tr>
<tr>
<td>Sodium Sulfapyridine</td>
</tr>
<tr>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>Brilliant Green</td>
</tr>
<tr>
<td>Phenol Red</td>
</tr>
</tbody>
</table>

**Difco™ SBG Sulfag Enrichment**

<table>
<thead>
<tr>
<th>Approximate Formula* Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast Extract</td>
</tr>
<tr>
<td>Peptone</td>
</tr>
<tr>
<td>D-Mannitol</td>
</tr>
<tr>
<td>Sodium Taurocholate</td>
</tr>
<tr>
<td>Sodium Sulfapyridine</td>
</tr>
<tr>
<td>Sodium Selenite</td>
</tr>
<tr>
<td>Dipotassium Phosphate</td>
</tr>
<tr>
<td>Monopotassium Phosphate</td>
</tr>
<tr>
<td>Brilliant Green</td>
</tr>
</tbody>
</table>

*Adjusted and/or supplemented as required to meet performance criteria.

**Uninoculated Plate**

Salmonella Typhimurium
ATCC™ 14028

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BD
User Quality Control

Identity Specifications

**Difco™ BG Sulfa Agar**

Dehydrated Appearance: Pink, free flowing, homogenous.

Solution: 5.9% solution, soluble in purified water upon boiling. Solution is very dark amber, very slightly to slightly opalescent.

Prepared Appearance: Orange-brown to dark reddish-amber, slightly opalescent.

Reaction of 5.9% Solution at 25°C: pH 6.9 ± 0.2

**SBG Sulfa Enrichment**

Dehydrated Appearance: Light beige, free-flowing, homogenous.

Solution: 2.42% solution, soluble in purified water. Solution is green, opalescent, may have a precipitate.

Prepared Appearance: Green, opalescent without significant precipitate.

Reaction of 2.42% Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

**Difco™ BG Sulfa Agar**

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>COLOR OF COLONIES/MEDIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>29212</td>
<td>10^2-2×10^3</td>
<td>None</td>
<td>--/no change</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>10^2-3×10^3</td>
<td>None to  poor</td>
<td>Yellow-green/Yellow-green</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>13315</td>
<td>10^2-3×10^3</td>
<td>None</td>
<td>--/no change</td>
</tr>
<tr>
<td>Salmonella enterica subsp. enterica serotype Enteritidis</td>
<td>13076</td>
<td>10^2-3×10^3</td>
<td>Good</td>
<td>Pink-white/red</td>
</tr>
<tr>
<td>Salmonella enterica subsp. enterica serotype Typhimurium</td>
<td>14028</td>
<td>10^2-3×10^3</td>
<td>Good</td>
<td>Pink-white/red</td>
</tr>
</tbody>
</table>

**SBG Sulfa Enrichment**

Prepare the enrichment per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, subculture onto plates of MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>ATCC</th>
<th>INOCULUM CFU</th>
<th>RECOVERY</th>
<th>COLOR ON MACCONKEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>25922</td>
<td>10^2-3×10^2</td>
<td>None to  poor</td>
<td>Pink, if any</td>
</tr>
<tr>
<td>Salmonella enterica subsp. enterica serotype Enteritidis</td>
<td>13076</td>
<td>10^2-3×10^2</td>
<td>Good</td>
<td>Colorless</td>
</tr>
<tr>
<td>Salmonella enterica subsp. enterica serotype Typhimurium</td>
<td>14028</td>
<td>10^2-3×10^2</td>
<td>Good</td>
<td>Colorless</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>9290</td>
<td>10^2-3×10^2</td>
<td>None to  fair</td>
<td>Colorless</td>
</tr>
</tbody>
</table>

Identity Specifications

**Difco™ SBG Sulfa Agar**

Dehydrated Appearance: Light beige, free-flowing, homogenous.

Solution: 2.42% solution, soluble in purified water. Solution is green, opalescent, may have a precipitate.

Prepared Appearance: Green, opalescent without significant precipitate.

Reaction of 2.42% Solution at 25°C: pH 7.2 ± 0.2

Expected Results

**BG Sulfa Agar**

The typical Salmonella colonies appear as pink-white to red opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense yellow-green zone. BG Sulfa Agar is not suitable for the isolation of S. Typhi or Shigella; however, some strains of S. Typhi may grow forming red colonies.

**SBG Sulfa Enrichment**

Examine prepared media for growth. Positive tubes should be subcultured onto prepared media for isolation and identification of bacteria.

Limitations of the Procedure

1. On BG Sulfa Agar colonies of Salmonella spp. vary from red to pink to white depending on length of incubation and strain.13
2. BG Sulfa Agar is normally orange-brown in color; however, on incubation, it turns bright red and returns to normal color at room temperature.13
3. S. Typhi does not grow adequately on BG Sulfa Agar. Shigella spp. do not grow on BG Sulfa Agar.13
4. Do not autoclave BG Sulfa Agar longer than 15 minutes; longer periods decrease the selectivity of the medium.

Directions for Preparation from Dehydrated Product

**Difco™ BG Sulfa Agar**

1. Suspend 59 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating, which will decrease selectivity.
4. Test samples of the finished product for performance using stable, typical control cultures.

**Difco™ SBG Sulfa Enrichment**

1. Suspend 24.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 5-10 minutes to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to appropriate references for specific procedures for the isolation and cultivation of Salmonella from meat, poultry and egg products and other foods.7,8

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5. Since BG Sulfa Agar is highly selective, it is recommended that less selective media, such as MacConkey Agar, be used simultaneously.

6. SBG Sulfa Enrichment should be used in conjunction with a selective prepared medium for bacterial identification.

References

Availability
Difco™ BG Sulfa Agar
CCAM COMPF USDA
Cat. No. 271710 Dehydrated – 500 g

Difco™ SBG Sulfa Enrichment
USDA
Cat. No. 271510 Dehydrated – 500 g