BRILLIANT GREEN SULFA AGAR

- For in vitro use only - Catalogue No. PB83

Our Brilliant Green Sulfa Agar is a selective and differential medium used in the isolation of *Salmonella* species from foods.

Brilliant Green Agar was initially developed in 1925 by Kristensen and later modified by Kauffmann in 1935. The sulfa-modification was described later by Osborn and Stokes. The base includes the nutritive components meat peptone, casein peptone, pancreatic digest of gelatin, and yeast extract that provide the organism with nitrogen, amino acids, vitamins, and other essential growth factors. Phenol red is the pH indicator that detects changes in pH due to the fermentation of sucrose and/or lactose. The selectivity is due to the presence of brilliant green and sulfadiazine. Brilliant green is a dye that inhibits the majority of gram-positive and gram-negative bacteria including *Salmonella typhi* and *Shigella* species. Sulfadiazine is a sulfonamide antibiotic that has both gram-positive and gram-negative activity. Inhibition of *E. coli* and *Proteus* species is markedly better on the sulfa-modification of Brilliant Green Agar thereby improving *Salmonella* isolation.

Formula per Litre of Medium

Casein / Meat Digests	6.0 g
Pancreatic Digests of Gelatin	8.0 g
Yeast Extract	3.0 g
Lactose	10.0 g
Sucrose	10.0 g
Sodium Chloride	5.0 g
Agar	20.0 g
Phenol Red	0.08 g
Brilliant Green	0.0125 g
Sulfadiazine	0.08 g

pH 6.9 ± 0.2

Recommended Procedure

Method A (General Use)

1. Allow medium to adjust to room temperature.
2. Streak the specimen directly on the agar surface as to obtain isolated colonies; or alternatively if a swab is being used, roll the swab over a small area near the edge of the plate and then proceed to streak from the inoculated area to the remainder of the plate.
3. A non-selective medium should also be streaked to increase the recovery of gram-negative organisms, and to characterize other organisms present in the sample.
4. Incubate plates at 35°C.
5. Examine plates after 24 and 48 hours.

Method B (HPB Procedure for Testing Food)

1. Weigh 25 g of food sample into a sterile blender jar and add 225mL of nutrient broth and blend. Incubate for 18-24 hours at 35°C.
2. Transfer 1 mL of the incubated nutrient broth to each tube of Tetrathionate Brilliant Green Broth (Dalynn TT32) and Selenite-Cysteine Broth (Dalynn TS46). Incubate both broths for 18-24 hours at 35°C.
3. Transfer a loopful (10µL) of each broth to separate plates of Bismuth Sulfite Agar (PB68) and Brilliant Green Sulfa Agar.
4. Incubate plates aerobically at 35°C.
5. Examine after 18-24 hours.
6. If no growth is observed re-incubate plates an additional 24 hours.

Interpretation of Results

On Brilliant Green Sulfa Agar, typical *Salmonella* colonies appear as pinkish-white or red colonies surrounded by a red halo in the medium. The red coloration in the medium

indicates that lactose or sucrose was not utilized. Differentiation is quite pronounced, as lactose or sucrose fermenting organisms, which are uninhibited or overcome inhibition, produce yellow-green colonies with a green halo.

Biochemical and/or serological tests should be performed on isolated colonies in order to complete identification.

- *Slow lactose fermenters such as Proteus or Pseudomonas may grow as red colonies*
- *Bacterial resistance to sulfonamides such as sulfadiazine is common therefore the growth of resistant organisms may be observed*
- *Not recommended for Shigella isolation*

Quality Control

After checking for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth performance of the completed medium.

Organism	Expected Result
Salmonella typhimurium ATCC 14028	Growth Pink with red halo
Escherichia coli ATCC 25922	Partial Inhibition Yellow-green with green halo

Storage and Shelf Life

Our Brilliant Green Sulfa Agar should be stored away from direct light at 4°C to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions this medium has a shelf life of 8 weeks from the date of manufacture.

References

1. Kristensen M., Lester V, Jurgens A. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br J Exp Pathol 1925; 6: 291-7.
2. Osborn WW, Stokes JL. The determination of salmonellae in foods. Ottawa: Food and Drug Laboratories, 1962.
3. Health Protection Branch. Methods for the isolation and identification of Salmonella from foods. In Government of Canada: Compendium of analytical methods, Vol 2. Morin Heights, Quebec: Polyscience Publications, 1997.
4. Difco Manual. 11th edition. Difco Laboratories: Maryland 1998.
5. Murray PR, Baron E, Pfaller M, Tenover F, Yolken R. Manual of Clinical Microbiology. 7th ed. Washington, DC: ASM, 1999.

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