



BISMUTH SULFITE AGAR

- For in vitro use only -

Catalogue No. PB68

Our Bismuth Sulfite Agar is a selective and differential medium used for the isolation of *Salmonella* species from a variety of samples including foods and clinical specimens.

Wilson and Blair published the initial formulation in 1926. Numerous researchers including Cope and Kasper, Gunther and Tuft, as well as Hajna and Perry validated the performance of the medium for isolating *Salmonella*. The high selectivity of the medium makes it ideal for food testing and is specified for the testing of dairy products including milk, cheese products, and butter. Efficacy has also been shown for the isolation of *Salmonella* from clinical samples.

The growth components of the medium include peptone, beef extract, and dextrose. Bismuth sulfite and brilliant green are general inhibitors that hinder the growth of most bacteria while allowing salmonellae to grow. Ferrous sulfate utilization by *Salmonella* species results in H₂S production and the formation of ferrous sulfide, an insoluble black precipitate. This precipitate gives *Salmonella* colonies their characteristic brown-black coloration and also diffuses into the surrounding medium.

Formula per Litre of Medium

Casein/Meat Peptone	10.0 g
Beef Extract.....	5.0 g
Dextrose	5.0 g
Dipotassium Phosphate.....	4.0 g
Ferrous Sulfate	0.3 g
Bismuth Sulfite.....	8.0 g
Agar	15.0 g
Brilliant Green.....	0.025 g

pH 7.7 ± 0.2

Recommended Procedure

1. Allow medium to adjust to room temperature prior to inoculation.
2. Using a direct inoculum from the specimen, perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate aerobically at 35°C.
4. Examine after 24 hours and 48 hours of incubation.

Interpretation of Results

A presumptive positive for *Salmonella* species is the isolation of dark gray to brown-black colonies with or without a metallic sheen. *Salmonella typhi* will appear as black colonies surrounded by brownish-black zones in the medium; a metallic sheen is normally present. Other *Salmonella* species have variable appearance; overnight coloration can vary from green to black, but uniformly black colonies are usually seen after 48 hours often with widespread staining of the medium and a metallic sheen.

Most other bacteria are inhibited but some bacteria may grow with the following appearance:

Shigella spp.: Inhibited but some strains may grow as brownish-green colonies with depressed centers

Proteus spp. and *E. coli*: Inhibited but some strains may grow as dull green or brown colonies with no metallic sheen or surrounding zones

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture in order to complete identification.

- *Atypical colonies may develop if medium is heavily contaminated with organic materials; to prevent this, suspend sample in sterile saline, centrifuge and use supernatant as a inoculum*
- *Typical Salmonella typhi colonies usually develop within 24 hours, but all plates should be incubated for 48 hours and examined prior to discarding*
- *Given the very limited shelf life of Bismuth Sulfite Agar plates, prepared plates are shipped prior to completion of our standard QC protocol*
- *Bismuth Sulfite Agar normally has an opalescent, greenish-brown coloration; prolonged storage (after 3 days) may result in a green color shift and a reduction in the selectivity of the medium*

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
<i>Salmonella typhimurium</i> ATCC 14028	Gray or brown-black colonies with or without metallic sheen
<i>Escherichia coli</i> ATCC 25922	Partial to complete inhibition

Storage and Shelf Life

Our Bismuth Sulfite Agar should be stored at 4°C to 8°C and protected from light. The medium side should be uppermost to prevent excessive accumulation of moisture on the agar surface. Under these conditions the medium has a shelf life of 7 days from the date of manufacture.

References

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