

Brilliant Green Agar Modified

Intended Use

Brilliant Green Agar Modified is used for isolating *Salmonella* from water, sewage and foodstuffs.

Summary and Explanation

Kampelmacher¹ proposed the formula for a selective medium to isolate *Salmonella* from pig feces and minced meat. Brilliant Green Agar Modified is more selective than Desoxycholate Citrate Agar and other brilliant green media, and inhibits the growth of *Pseudomonas aeruginosa* and partially inhibits the growth of *Proteus* spp. which may resemble *Salmonella*.

Salmonella enterica grows well on Brilliant Green Agar Modified compared to Desoxycholate Citrate Agar.²

Brilliant Green Agar Modified is recommended for the isolation of *Salmonella*, other than *Salmonella* Typhi, from water and associated materials³ and meat and meat products.⁴ It is recommended by the British Poultry Meat Society⁵ for the examination of poultry and poultry products. The recommended procedures include using complementary selective culture media and techniques to increase the likelihood of isolating multiple serotypes of *Salmonella* from samples.⁶

User Quality Control

Identity Specifications

Difco™ Brilliant Green Agar Modified

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 5.2% solution, soluble in purified water upon boiling. Solution is orange-brown, clear to slightly opalescent.

Prepared Appearance: Orange-brown, clear to slightly opalescent.

Reaction of 5.2%

Solution at 25°C: pH 6.9 ± 0.1

Cultural Response

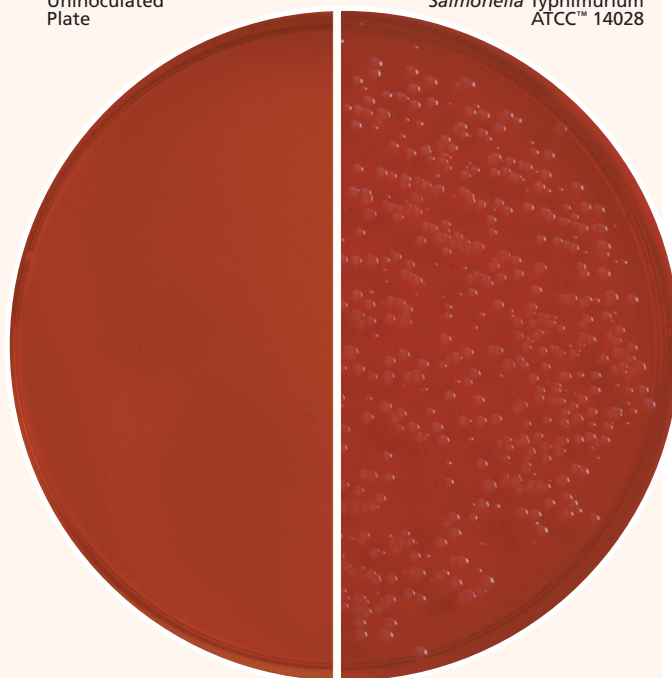
Difco™ Brilliant Green Agar Modified

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Escherichia coli</i>	25922	10 ³	Complete to partial inhibition	Green
<i>Proteus mirabilis</i>	25933	10 ³	Complete to partial inhibition	Red
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ² -10 ³	Good	Red

Uninoculated
Plate

Salmonella Typhimurium
ATCC™ 14028



Principles of the Procedure

Brilliant Green Agar Modified contains beef extract and peptone as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Lactose and sucrose are carbohydrate sources. In the presence of phenol red, a pH indicator, lactose- and/or sucrose-nonfermenting *Salmonella* will produce red colonies. Brilliant green inhibits gram-positive organisms and many gram-negative bacteria, except *Salmonella*. Agar is the solidifying agent.

Formula

Difco™ Brilliant Green Agar Modified

Approximate Formula* Per Liter

Beef Extract.....	5.0	g
Peptone	10.0	g
Yeast Extract	3.0	g
Disodium Phosphate	1.0	g
Monosodium Phosphate	0.6	g
Lactose	10.0	g
Sucrose	10.0	g
Phenol Red.....	0.09	g
Brilliant Green	4.7	mg
Agar	12.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 52 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. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Meat and Meat Products

1. Weigh 25 g of the sample into a sterile blender jar and add 225 mL of Buffered Peptone Water. Macerate for a sufficient time to give 15,000-20,000 revolutions.
2. Aseptically transfer the contents of the blender jar to a 500 mL flask. Incubate at $37 \pm 0.1^\circ\text{C}$ for 16-20 hours.
3. Transfer 10 mL samples to 100 mL Muller Kauffmann Tetrathionate Broth.
4. Incubate the Muller Kauffmann Tetrathionate Broth at $42-43^\circ\text{C}$.

Sewage Polluted Natural Water

This procedure is applicable to the isolation of *Salmonella* spp. other than *S. Typhi*.

1. Inoculate 25 mL aliquots of the sample into 25 mL of double strength Buffered Peptone Water and incubate at 37°C for 18 hours.
2. Transfer 1 mL samples into 10 mL of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.

Subculture

1. Subculture from the broth at 18-24 hours and at 48 hours onto Brilliant Green Agar Modified.
2. Examine for typical colonies of *Salmonella* after overnight incubation at 37°C .

Expected Results

Salmonella will produce red colonies.

Limitations of the Procedure

1. Organisms other than *Salmonella* spp., such as *Morganella morganii* and some *Enterobacteriaceae*, may grow on the medium.
2. Confirmatory tests, such as fermentation reactions and seroagglutination, should be carried out on all presumptive *Salmonella* spp.

References

1. Guinee and Kampelmacher. 1962. *Antonie van Leeuwenhoek* 28:417.
2. Heard, Jennet and Linton. 1969. *Br. Vet. J.* 125:635.
3. H. M. S. O. 1982. Methods for the isolation and identification of salmonellae (other than *Salmonella typhi*) from water and associated materials.
4. International Organisation for Standardization. 1974. Draft International Standard ISO/DIS 3565. Geneva, Switzerland.
5. British Poultry Meat Society. 1982. A manual of recommended methods for the microbiological examination of poultry and poultry products.
6. Harvey and Price. 1976. *J. Hyg. Camb.* 77:333.

Availability

Difco™ Brilliant Green Agar Modified

ISO

Cat. No. 218801 Dehydrated – 500 g

Europe

Cat. No. 254490 Prepared Plates – Pkg. of 20*

*Store at $2-8^\circ\text{C}$.