

Brilliant Green Agar

Intended Use

This medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Brilliant Green Agar is a highly selective medium for the isolation of *Salmonella* other than *S. typhi* from feces and other materials.

Summary and Explanation

Brilliant Green Agar was first described by Kristensen et al. in 1925.¹ Their formulation was modified slightly by Kauffmann in 1935.² The medium is included in procedures for the examination of water and wastewater.³ The medium is also included in the *USP* for use in the performance of Microbial Limit Tests.⁴ It is classified as a highly selective medium for the recovery of salmonellae except for the typhoid and paratyphoid bacilli.⁵

Principles of the Procedure

Brilliant green dye inhibits gram-positive bacteria and a majority of gram-negative bacilli. Phenol red serves as a pH indicator and yields a yellow color as a result of acid production in the fermentation of the lactose and/or sucrose in the medium.

Formula

Difco™ Brilliant Green Agar

Approximate Formula* Per Liter		
Proteose Peptone No. 3	10.0	g
Yeast Extract	3.0	g
Lactose	10.0	g
Saccharose	10.0	g
Sodium Chloride	5.0	g
Agar	20.0	g
Brilliant Green	12.5	mg
Phenol Red	0.08	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 58 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.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A less selective medium and a nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

User Quality Control

Identity Specifications

Difco™ Brilliant Green Agar

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in purified water upon boiling. Solution is brownish-green, clear to very slightly opalescent.
Prepared Appearance:	Orange-brown, very slightly to slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ Brilliant Green Agar

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 ⁴	Poor	Yellow-green
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Enteritidis	13076	30-300	Good	Red
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhi	19430	30-300	None to poor	Red
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	30-300	Good	Red
<i>Staphylococcus aureus</i>	25923	~10 ⁴	Marked inhibition	–

Expected Results

Typical colonial morphology on Brilliant Green Agar is as follows:

<i>Salmonella</i> (other than <i>S. typhi</i> and <i>S. paratyphi</i>)	White to red, opaque colonies surrounded by red zones in the medium
<i>S. typhi</i> and <i>S. paratyphi</i>	No growth to trace growth
<i>Shigella</i>	No growth to trace growth
<i>Escherichia coli</i> and <i>Enterobacter/Klebsiella</i>	Yellow to greenish-yellow colonies surrounded by intense yellow-green zones in medium
<i>Proteus</i>	No growth to trace growth
<i>Pseudomonas</i>	Pink to red colonies
Gram-positive bacteria	No growth to trace growth

References

1. Kristensen, Lester and Jurgens. 1925. Br. J. Exp. Pathol. 6:291.
2. Kauffmann. 1935. Z. Hyg. Infektionskr. 117:26.
3. Downes and Ito (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
4. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.

Availability

Difco™ Brilliant Green Agar

EP SMWW USP

Cat. No. 228530 Dehydrated – 500 g

BBL™ Brilliant Green Agar

EP SMWW USP

Cat. No. 295963 Prepared Plates – Pkg. of 20*

*Store at 2-8°C.

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 choleraesuis* 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

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.

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 choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 ² -10 ³	Good	Red

Uninoculated Plate

Salmonella typhimurium ATCC™ 14028

