

# Brilliant Green Agar

## Intended Use

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.<sup>1</sup> Their formulation was modified slightly by Kauffmann in 1935.<sup>2</sup> The medium is included in procedures for the examination of water and wastewater.<sup>3</sup>

## 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.

## References

1. Kristensen, Lester and Jurgens. 1925. Br. J. Exp. Pathol. 6:291.
2. Kauffmann. 1935. Z. Hyg. Infektionskr. 117:26.
3. Eaton, Rice and Baird (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed., online. American Public Health Association, Washington, D.C.

## 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

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

## Availability

### Difco™ Brilliant Green Agar

EP SMWW

Cat. No. 228530 Dehydrated – 500 g

### BBL™ Brilliant Green Agar

EP SMWW

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

\*Store at 2-8°C.