**TECHNICAL DATA SHEET**

# BRILLIANT GREEN (KRISTENSEN)

**DETECTION OF *SALMONELLA***

## 1 INTENDED USE

Brilliant Green Agar of Kristensen is a highly selective medium used to isolate salmonella, except for *Salmonella* Typhi, in biological samples of animal origin and food products.
The agar can also be used as the second media of choice in the normalized standards for the research and detection of *Salmonella*.

## 2 HISTORY

In 1925, Kristensen, Lester and Jurgens described this medium, later modified by Kauffmann and then used by Broh-Kahn and Edwards with satisfaction.

## 3 PRINCIPLES

The inhibition of Gram-positive flora and most Gram-negative bacteria is due to the presence of brilliant green.

The medium contains two carbohydrates, lactose and sucrose, for which fermentation of either or both results in a decrease in pH, causing the appearance of yellow-green colonies in the presence of the pH indicator, phenol red. Salmonella produces colorless to pinkish colonies.

It is particularly recommended to simultaneously inoculate enrichment broths onto other less selective media such as MacConkey agar, XLD agar, or Hektöen agar.

Due to its high inhibitory power, this medium allows the use of a heavily contaminated inoculum.

## 4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Tryptone .....5.0 g
- Peptic digest of meat .....5.0 g
- Yeast extract.....3.0 g
- Lactose .....10.0 g
- Sucrose .....10.0 g
- Sodium chloride.....5.0 g
- Phenol red .....80.0 mg
- Brilliant green.....12.5 mg
- Bacteriological agar .....13.5 g

pH of the ready-to-use media at 25 °C : 6.9 ± 0.2.

## 5 PREPARATION

- Dissolve 51.6 g of dehydrated media (BK071) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes of vials at 100 mL per vial.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47 °C.
- Pour into sterile Petri plates.
- Let cool on a cold, flat surface.
- Dry the plates in an incubator with the covers partially removed.

✓ **Reconstitution :**
**51.6 g/L**

✓ **Sterilization :**
**15 min at 121 °C**

## 6 INSTRUCTIONS FOR USE

- Inoculate by streaking on plates, using the enrichment media as inoculum.
- Incubate at 37 °C for 24 to 48 hours.

✓ **Inoculation :**
**Surface streaking**

✓ **Incubation :**
**24 to 48 h at 37 °C**

**NOTE**

The medium, normally red-brown, becomes bright red after incubation and assumes its original color after returning to room temperature.

## 7 RESULTS

The colonies have the following appearance :

| Characteristics | Microorganisms |
|---|---|
| Colorless to pink colonies, smooth, surrounded by red zones in the media | *Salmonella* lactose/sucrose-negative with the exception of *Salmonella* Typhi & Paratyphi. |
| No or very weak growth | *Shigella* |
| Yellow to green colonies, surrounded by yellow green zones in the media | *Escherichia coli, Citrobacter, Klebsiella, Enterobacter* (lactose/sucrose positive) |
| Totally or almost totally inhibited | Gram positive bacteria |

## 8 QUALITY CONTROL

**Dehydrated media** : pinkish powder, free-flowing and homogeneous.
**Prepared media** : orange-brown agar.

Typical culture response after 48 hours of incubation at 37 °C, qualitative method of inoculation :

| Microorganisms | | Growth | Characteristics |
|---|---|---|---|
| *Salmonella* Typhimurium | WDCM 00031 | Good, score 2 | Pink colonies |
| *Salmonella* Enteritidis | WDCM 00030 | Good, score 2 | Pink colonies |
| *Escherichia coli* | WDCM 00013 | Good, score 2 | Yellow colonies |
| *Staphylococcus aureus* | WDCM 00034 | Inhibited, score 0 | - |

## 9 STORAGE / SHELF LIFE

**Dehydrated media :** 2-30 °C.
The expiration date is indicated on the label.

**Prepared media in vials (*) :** Not recommended, do not re-melt the media.
**Prepared media in plates (*) :** 30 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## 10 PACKAGING

**Dehydrated media :**
500 g bottle..... BK071HA

## 11 BIBLIOGRAPHY

Kristensen, M., Lester, V., and Jurgens, A.. 1925. On the use of trypsinized casein, brom-thymol-blue, brom-cresol-purple, phenol-red and brilliant-green for bacteriological nutrient media. British Journal of Experimental Pathology, **6** : 291-299.

NF EN ISO 11133. July 2014. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

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## 12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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