IFU

Instructions for Use

BRILLIANT GREEN AGAR WITH NOVOBIOCIN

Cat. no. G175	Brilliant Green Agar with Novobiocin, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. J127BX	XLT-4 Agar/Brilliant Green Agar with Novobiocin, 15x100mm Biplate, 10ml/10ml	100 plates/box

INTENDED USE

Hardy Diagnostics Brilliant Green Agar with Novobiocin is recommended for the selective isolation of *Salmonella* spp., other than S. Typhi, from non-clinical samples.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Brilliant Green Agar, Modified was formulated by Edel and Kamplemacher of the Netherlands Institute for Public Health, Utrecht and originally proposed as a selective medium for the isolation of *Salmonella* from pig feces and minced meat.[8,9] The medium has been widely utilized in Europe and used in the Standard European Community and by the International Standards Organization.[7,10] More selective than Deoxycholate Citrate Agar and other brilliant green media, Brilliant Green Agar, Modified inhibits the growth of *Pseudomonas aeruginosa* and *Proteus* spp., which may resemble the growth of *Salmonella* spp., thus facilitating the isolation of *Salmonella* colonies.

Brilliant Green Agar, Modified is recommended for the selective isolation of *Salmonella* spp., other than S. Typhi, from clinical and non-clinical specimens. The medium is appropriate for subculture from selective enrichment media. The addition of novobiocin to the medium reduces the incidence of nuisance flora commonly cultured in conjunction with *Salmonella*.

FORMULA

Ingredients per liter of deionized water:*

Lactose	10.0gm
Sucrose	10.0gm
Casein Peptone	5.0gm
Animal Tissue Peptone	5.0gm
Gelatin Peptone	5.0gm
Sodium Chloride	5.0gm
Yeast Extract	3.0gm
Disodium Phosphate	1.0gm

Phenol Red	0.09gm
Monopotassium Phosphate	0.52gm
Brilliant Green	4.7gm
Novobiocin	2.0ml
Agar	12.0gm

Final pH 6.9 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

For Meat and Meat Products :

1. Weigh out 25.0gm of the sample into a sterile blender.

2. Add 225ml of Buffered Peptone Water (Cat. no. U142) to the blender and macerate the sample and buffer to homogenize the mixture to yield 15,000 to 20,000 revolutions.

3. Asceptically transfer the contents of the blender jar to a sterile 500ml flask and incubate at 35 to 37ºC. for 16 to 20 hours.

4. Transfer 10ml samples to 100ml of Tetrathionate Broth Base (Cat. no. U165) supplemented with Iodine-Iodide Solution (Cat. no. Z129 or Z139).

5. Incubate at 42 to 43ºC. for 48 hours.

For Sewage and Polluted Water : (for *Salmonella* spp. other than S. Typhi)

1. Inoculate 25ml aliquotes of the sample into 25ml of double strength Buffered Peptone Water and incubate samples at 35 to 37 ºC. for 18 hours.

2. Transfer 1ml samples into 10ml of Tetrathionate Broth Base (Cat. no. K65) supplemented with Iodine-Iodide Solution (Cat. no. Z129 or Z139)

3. Incubate at 43ºC. for 48 hours.

Subculture:

1. Subculture from the Tetrathionate Broth at 18 to 24 hours and at 48 hours onto Brillian Green Agar, Modified.

2. Incubate plates at 35 to 37ºC. for 18 to 24 hours.

3. Examine plates for typical *Salmonella* colony morphology.

INTERPRETATION OF RESULTS

Typical *Salmonella* colonies will produce red to pink-white with red zones. The red coloration of the medium indicates that lactose or sucrose was not utilized.

Lactose or sucrose fermenting microorganisms not completely inhibited by the medium will show as yellow to yellow-green colonies with a yellow-green or green zone.

Other non-lactose fermenting microorganisms may mimic enteric pathogens and present as red to pink-white colonies surrounded by red zones. Further biochemical testing is needed to fully identify these strains.

Escherichia coli may be partially inhibited and present as yellow to yellow-green colonies with a green halo.

Shigella spp. may exhibit partial to complete inhibition with colorless colonies.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Organisms other than *Salmonella* spp., such as *Morganella morgani* and some *Enterobacterales*, may grow on this medium.

Fermentation reactions, seroagglutination and other confirmatory tests should be carried out on all colonies that are presumptive for *Salmonella*.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, other culture media such as Buffered Peptone Water (Cat. no. U142), Tetrathionate Broth Base (Cat. no. K65 or U165), as well as supplements, such as Iodine-Iodide Solution (Cat. no. Z129 or Z139), etc., are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth; red to pink-white colonies with red zones
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial inhibition; small yellow to yellow-green colonies
Staphylococcus aureus ATCC® 25923	B	18-24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Brilliant Green Agar with Novobiocin should appear slightly opalescent, and brownish-orange in color.

REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

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4. Anon. 1981. Microbiology - General Guidance on Methods for the Detection of *Salmonella*. Ref. method ISO 6579-1981(E). International Organization for Standardization. Geneva, Switzerland.

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8. Edel, W. and E.H. Kamplemacher. 1968. Comparative studies on Salmonella isolation in eight European laboratories. *Bull. Wld. Hlth. Org*.; 39:487-491.

9. Edel, W. and E.H. Kamplemacher. 1969. *Salmonella* infections in nine European lanoratories using a standard technique. *Bull. Wld. Hlth. Org*.; 41:297-306.

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