

1. Product Identification

Product Name	Bromocresol Purple Azide Broth
Catalogue Number	AS-1154
Document Reference	TDS-AS-1154-v1
Issue Date	April 2026
Medium Type	Selective Enrichment Liquid Broth
Category	Water & Food Microbiology

2. Composition (per litre)

Ingredient	CAS No.	g/L
Tryptose	91079-38-8	20.0
Yeast Extract	8013-01-2	5.0
Dextrose (D-Glucose)	50-99-7	1.0
Dipotassium Hydrogen Phosphate (K ₂ HPO ₄)	7758-11-4	2.7
Bromocresol Purple	115-40-2	0.016
Sodium Azide (NaN ₃)	26628-22-8	0.4
Total (approx.)		~29.1

3. Preparation Instructions

1. Weigh approximately 29.1 g of dehydrated powder per litre of purified (distilled or deionised) water.
2. Suspend the powder in approximately 750 mL water with constant stirring.
3. Heat gently with frequent agitation and bring to the boil to dissolve completely.
4. Make up to 1 litre with water if volume was reduced during boiling.
5. DO NOT AUTOCLAVE. Sterilise by boiling for exactly 10 minutes.
6. Cool to 45–50 °C. Dispense aseptically into sterile Durham tubes or culture vessels.
7. Allow to cool to room temperature. Verify pH = 6.8 ± 0.2 at 25 °C.

⚠ WARNING: Autoclave treatment destroys sodium azide selectivity and degrades bromocresol purple. Boiling 10 min is the only validated sterilisation method.

4. Mode of Action

Sodium Azide (NaN₃) at 0.4 g/L selectively inhibits the cytochrome c oxidase enzyme complex in Gram-negative bacteria, blocking the aerobic respiratory chain and causing cell death. Catalase-negative enterococci and streptococci, which lack functional cytochrome oxidase, are resistant to these effects and grow freely.

Bromocresol Purple (sulphonaphthalein dye, pKa ≈ 6.0) functions as a pH indicator: purple/violet above pH 6.8; yellow below pH 5.2. Acid produced by enterococcal fermentation of glucose (primarily via

homofermentative glycolysis to lactic acid) drives this colour transition, constituting the presumptive positive indicator.

5. Inoculation & Incubation

Technique	Multiple tube fermentation (MPN) or membrane filtration enrichment
Inoculum Volume	1 mL or 0.1 mL of sample per tube (per method requirements)
Incubation Temperature	35–37 °C
Incubation Period	24–48 hours; read at 24 h; re-incubate to 48 h if negative
Atmosphere	Aerobic (cap loosened slightly to allow gaseous exchange)
Positive Result	Yellow colour change and/or turbidity in tube
Negative Result	Purple colour maintained; no turbidity
Confirmation	Positive tubes should be confirmed on selective solid media (e.g., Aesculin Azide Agar or KF Streptococcus Agar)

6. Quality Control

Organism	ATCC No.	Expected Result	Growth / Inhibition	CFU Inoculum
Enterococcus faecalis	ATCC 29212	Growth + yellow colour	Growth (positive)	≤100 CFU/tube
Enterococcus faecium	ATCC 19434	Growth + yellow colour	Growth (positive)	≤100 CFU/tube
Streptococcus bovis	ATCC 9809	Growth + yellow colour	Growth (positive)	≤100 CFU/tube
Escherichia coli	ATCC 25922	Inhibited; no colour change	Inhibited	≤100 CFU/tube
Klebsiella pneumoniae	ATCC 13883	Inhibited; no colour change	Inhibited	≤100 CFU/tube

7. Performance Standards

- ISO 7899-1:1998 — Water quality: Detection and enumeration of intestinal enterococci (MPN method)
- APHA Standard Methods, 23rd Ed. — Method 9230B
- AS/NZS 4276.9 — Water microbiology: Enterococci
- APHA Compendium of Methods for the Microbiological Examination of Foods (4th Ed.)

8. Storage & Shelf Life

Dehydrated Powder	Store at 10–30 °C in a dry location, protected from light and moisture. Shelf life: 3 years from manufacture date when stored correctly.
Prepared Broth	Store at 2–8 °C. Use within 2 weeks. Discard if colour change occurs before inoculation.

Indicator of Deterioration

Yellow colour in uninoculated prepared broth indicates deterioration.
Do not use.

9. Literature References

1. Mallmann, W.L. & Seligmann, E.B. (1950). A comparative study of media for the detection of streptococci in water and sewage. *American Journal of Public Health*, 40, 286–289.
2. ISO 7899-1:1998. Water quality — Detection and enumeration of intestinal enterococci — Part 1: Miniaturized method (MPN). ISO, Geneva.
3. APHA (2017). *Standard Methods for the Examination of Water and Wastewater*, 23rd Ed., Method 9230B. American Public Health Association, Washington DC.
4. Murray, P.R. et al. (2007). *Manual of Clinical Microbiology*, 9th Ed. ASM Press, Washington DC.
5. Holt, J.G. et al. (1994). *Bergey's Manual of Determinative Bacteriology*, 9th Ed. Williams & Wilkins, Baltimore.
6. Facklam, R.R. & Collins, M.D. (1989). Identification of *Enterococcus* species isolated from human infections. *Journal of Clinical Microbiology*, 27(4), 731–734.

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