



TDS
AS-1397

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R2A Broth

Reasoner's 2A Broth — Low-Nutrient, Non-Selective Liquid Medium | Catalogue No. AS-1397

1. Product Overview

R2A Broth (Reasoner's 2A Broth) is a low-nutrient, non-selective liquid culture medium originally formulated by Reasoner and Geldreich (1985) for the recovery and cultivation of heterotrophic bacteria from treated drinking water and environmental samples. Its deliberately diluted nutrient composition supports the growth of oligotrophic, stressed, and slow-growing microorganisms that are frequently inhibited on conventional nutrient-rich media. AS-1397 is the internationally recognised broth format for heterotrophic plate count (HPC) enrichment, Most Probable Number (MPN) assays, biofilm studies, and environmental microbial ecology. It is validated per APHA Standard Methods 9215, US EPA Method 9215, ISO 8245, and ASTM D9217.

2. Product Specifications

Format	Dehydrated powder — reconstitute as liquid broth
Medium Type	Low-nutrient, non-selective liquid medium
Final pH (prepared, 25°C)	7.2 ± 0.2
Suspension Rate	3.12 g/L
Sterilisation	Autoclave at 121°C for 15 minutes. Do not over-autoclave.
Appearance (powder)	Cream to yellow, homogeneous, free-flowing powder
Appearance (prepared)	Yellow-coloured, clear solution
Intended Use	For Laboratory Use Only — Not for human, animal, or food use

3. Composition — per litre of prepared medium

Ingredient	g/L	Function
Proteose Peptone	0.500	Complex nitrogen, amino acid, and growth factor source
Casein Acid Hydrolysate (Casamino Acids)	0.500	Defined amino acid mixture; supports fastidious organisms
Yeast Extract	0.500	B-vitamins, nucleotides, and accessory growth factors
Dextrose (D-Glucose)	0.500	Readily metabolisable carbon and energy source
Soluble Starch	0.500	Neutralises toxic metabolites; mimics environmental polysaccharides

Ingredient	g/L	Function
Dipotassium Phosphate (K_2HPO_4)	0.300	Phosphate buffer and essential phosphorus source
Sodium Pyruvate	0.300	Rapidly catabolised carbon source; supports stressed-cell recovery
Magnesium Sulphate	0.024	Essential magnesium cofactor; ion source
Purified Water	q.s. 1 L	—

Total: 3.12 g/L | Final pH 7.2 ± 0.2 at 25°C. Formula adjusted and standardised to suit performance parameters.

4. Preparation Protocol

- Dissolve 3.12 g in 1 L of purified (Milli-Q or Type 1) water with gentle agitation at room temperature. Heat if necessary to fully dissolve.
- Dispense into flasks, tubes, or containers as required.
- Autoclave at 121°C for 15 minutes. Do not over-autoclave — excessive heat will caramelise carbohydrate components, causing browning and performance degradation.
- Cool to 20–28°C before inoculation. Inspect: yellow-coloured, clear solution expected.
- Neutralise residual disinfectants in water samples (e.g. 0.1 mL of 3% sodium thiosulfate per 100 mL sample) before inoculation.

5. Incubation & Interpretation

Temperature	20–28°C (environmental/water organisms); 35–37°C for faecal indicators
Duration	24–72 hours for faster-growing strains; up to 7 days for field/environmental water samples
Positive Result	Turbidity, visible biomass, pellicle, or sediment formation
Negative Result	Clear broth throughout full incubation period
Further Testing	Plate onto R2A Agar; Gram stain; microscopy; molecular identification

6. Performance Specifications

Parameter	Specification	Method
Sterility (prepared)	No turbidity after 14 d at 28°C	USP <71>
Productivity — <i>E. coli</i> ATCC 25922	Good–luxuriant growth, 24–72 h at 35–37°C	APHA 9215 / ISO 8245
Productivity — <i>Enterococcus faecalis</i> ATCC 29212	Good–luxuriant growth, 24–72 h at 35–37°C	APHA 9215 / ISO 8245



Parameter	Specification	Method
Productivity — Salmonella Enteritidis ATCC 13076	Good–luxuriant growth, 24–72 h at 35–37°C	APHA 9215 / ISO 8245
Moisture Content (powder)	< 5.0%	Loss on drying, 105°C

7. Key Limitations

- Non-selective — strict aseptic technique is essential to avoid environmental contamination.
- May not support growth of copiotrophic (high-nutrient-requiring) organisms.
- Residual disinfectants (chlorine, chloramine) in water samples will inhibit recovery — neutralise before inoculation.
- Turbidity alone is not definitive; assess all visual cues and use uninoculated controls.
- Over-autoclaving will degrade carbohydrate components.

8. Storage & Shelf Life

Dehydrated Powder	Below 30°C; dry; original tightly sealed container
Prepared Broth	2–8°C; protected from light; use within 4 weeks
Shelf Life (powder)	As stated on label (typically 3 years from manufacture)

9. Applications

- Heterotrophic plate count (HPC) enrichment — drinking water testing, APHA 9215
- Most Probable Number (MPN) assays
- Biofilm and suspension culture studies
- Environmental microbial ecology and diversity studies

10. Literature & References

1. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. Applied and Environmental Microbiology. 1985;49(1):1–7.
2. Standard Methods for the Examination of Water and Wastewater, 24th ed. APHA, AWWA, WEF.
3. ISO 8245:1999. Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC).
4. ASTM D9217. Standard Test Method for Heterotrophic Plate Count.



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