

Marine Broth 2216 (Zobell Marine Broth)

Universal enrichment broth for marine heterotrophic bacteria.

OVERVIEW

Marine Broth 2216, originally formulated by Claude E. Zobell in 1941 at the Scripps Institution of Oceanography, is the most widely referenced liquid medium in marine microbiology. It was designed to simulate the nutritional and ionic environment of seawater, enabling the cultivation of heterotrophic marine bacteria that fail to grow on conventional terrestrial media.

The medium provides a low-organic-load nutritional base (peptone + yeast extract) in a complex marine salt system. The deliberate low nutrient concentration reflects the oligotrophic nature of many marine environments and prevents the overgrowth of fast-growing terrestrial contaminants, making it well suited for the recovery of authentic marine isolates from seawater, sediment, coral, fish tissue, and aquaculture samples.

PRODUCT IDENTIFICATION

Product Name	Marine Broth 2216 (Zobell Marine Broth)
Catalogue Number	AS-1290
Medium Type	Non-selective enrichment broth — marine
Solid Counterpart	Marine Agar 2216 (AS-1289)
Reference Formula	Zobell CE, 1941; BD Difco Marine Broth 2216 (Cat. 216000)
Dissolution	37.4 g/L in natural or artificial seawater (or distilled water)
Final pH (25°C)	7.6 ± 0.2
Sterilisation	Autoclave 121°C for 15 minutes
Prepared Broth Appearance	Clear to slightly hazy, light amber
Dehydrated Appearance	Cream to light beige free-flowing powder

MODE OF ACTION

The comprehensive salt system (NaCl, MgCl₂, MgSO₄, CaCl₂, KCl, NaHCO₃, and trace minerals) replicates the ionic composition of natural seawater (~35 g/L salinity), providing the osmotic conditions essential for the growth of obligate and facultative marine bacteria. Peptone supplies amino acids and nitrogen; yeast extract provides B-vitamins and accessory growth factors. Ferric citrate (0.1 g/L) supplies bioavailable iron — a limiting nutrient in ocean surface waters — supporting siderophore production, pigmentation, and iron-dependent metabolic pathways in marine isolates.

FORMULA — PER LITRE (ZOBELL 1941 / BD DIFCO STANDARD)

Component	g/L	Role
Peptone	5.000	Nitrogen, carbon, peptides — primary nutrient source
Yeast Extract	1.000	B-vitamins, nucleotides, accessory growth factors
Ferric Citrate	0.100	Bioavailable iron — siderophore support, pigmentation
Sodium Chloride	19.450	Primary osmotic salt — seawater salinity

Magnesium Chloride	5.900	Mg ²⁺ ion source
Magnesium Sulfate	3.240	SO ₄ ²⁻ source — seawater sulfate balance
Calcium Chloride	1.800	Ca ²⁺ ion source
Potassium Chloride	0.550	K ⁺ ion source
Sodium Bicarbonate	0.160	Buffering — carbonate/bicarbonate equilibrium
Potassium Bromide	0.080	Trace halide — seawater ionic mimic
Strontium Chloride	0.034	Trace mineral
Boric Acid	0.022	Trace mineral
Disodium Phosphate	0.008	Phosphorus source
Sodium Silicate	0.004	Trace mineral
Sodium Fluoride	0.0024	Trace halide
Ammonium Nitrate	0.0016	Trace nitrogen

Total: 37.4 g/L. Final pH 7.6 ± 0.2 at 25°C. Note: Marine Broth 2216 uses Magnesium Chloride (5.9 g/L) and Magnesium Sulfate (3.24 g/L); this differs from Marine Agar 2216, which uses Magnesium Chloride (8.8 g/L) and Sodium Sulfate (3.24 g/L) — the two products are not identical formulas despite sharing a name.

PREPARATION PROTOCOL

Step	Instruction
1	Suspend 37.4 g of dehydrated Marine Broth 2216 (AS-1290) in 1 litre of natural or artificial seawater (or distilled water if the dehydrated salt components are included in the formula).
2	Mix with gentle warming (45°C) until powder is completely dissolved. The prepared broth should appear clear to slightly hazy, light amber.
3	Adjust pH to 7.6 ± 0.2 if required using 1M NaOH or 1M HCl.
4	Dispense into tubes, flasks, or bottles as required.
5	Autoclave at 121°C for 15 minutes. Do not over-autoclave — excessive heating causes caramelisation and colour change.
6	Cool to inoculation temperature. For most marine heterotrophs, incubate at 15–25°C for 24–72 hours. For pathogenic vibrios, incubate at 35–37°C.

Seawater note: Natural aged seawater is preferred for optimal recovery of genuine marine isolates. Artificial seawater (e.g. ASTM D1141 formula) is a suitable alternative. If preparing synthetic seawater, ensure MgSO₄ is used as the sulfate source for this broth formula.

INCUBATION PARAMETERS

Organism Type	Temperature	Duration	Expected Result
General marine heterotrophs	20–25°C	24–72 h	Turbid growth
Psychrophilic marine bacteria	10–15°C	48–96 h	Turbid to slight growth
Pathogenic <i>Vibrio</i> spp.	35–37°C	18–24 h	Heavy turbid growth
Slow-growing oligotrophs	15–20°C	7–14 days	Slight turbidity

TARGET ORGANISMS

Organism / Group	Growth	Notes
<i>Vibrio</i> spp. (non-pathogenic)	Excellent	<i>V. harveyi</i> , <i>V. alginolyticus</i> , <i>V. splendidus</i> and related species

Photobacterium spp.	Excellent	Bioluminescent marine bacteria
Pseudoalteromonas spp.	Excellent	Common marine heterotroph
Shewanella spp.	Very good	Iron-reducing marine bacteria
Marinomonas spp.	Very good	Marine alphaproteobacteria
Alteromonas spp.	Very good	Aerobic marine heterotrophs
Marine halophiles	Good	Requires correct salinity
Slow-growing oligotrophs	Moderate	Extended incubation (7–14 days) may be required

STORAGE & STABILITY

Dehydrated Powder	Store at 10–30°C in a tightly sealed container, away from moisture and light. Desiccant recommended.
Prepared Broth	2–8°C, use within 3 months. Discard if turbid prior to inoculation.
Shelf Life	Refer to expiry date on label.

LITERATURE REFERENCES

1. Zobell CE. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J Mar Res. 1941;4(1):42–75.
2. BD Difco & BBL Manual of Microbiological Culture Media, 2nd ed. Becton Dickinson; 2009. Marine Broth 2216, Cat. 216000.
3. Austin B, Austin DA. Bacterial Fish Pathogens: Disease of Farmed and Wild Fish, 5th ed. Springer; 2012.
4. Kester DR, Duedall IW, Connors DN, Pytkowicz RM. Preparation of artificial seawater. Limnol Oceanogr. 1967;12(1):176–179.
5. Lyman J, Fleming RH. Composition of sea water. J Mar Res. 1940;3:134–146.

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