

Marine Agar 2216 • Marine Broth 2216

Intended Use

Marine Agar 2216 and Marine Broth 2216 are used for cultivating heterotrophic marine bacteria.

Summary and Explanation

Marine bacteria are present in nutrient sea water by the millions per mL and are essential to the life cycle of all marine flora and fauna. The enumeration and activity of marine bacteria are important to the food industry for the conservation of marine life. Marine Agar 2216 and Marine Broth 2216 are prepared according to the formula of ZoBell¹. The media contain all of the nutrients necessary for the growth of marine bacteria.

User Quality Control

Identity Specifications

Difco™ Marine Agar 2216

Dehydrated Appearance: Light beige with a few dark particles, free flowing, homogeneous.

Solution: 5.51% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent to opalescent with slight precipitate.

Prepared Appearance: Light amber, slightly opalescent to opalescent, may have a slight precipitate, may contain dark particles.

Reaction of 5.51%
Solution at 25°C: pH 7.6 ± 0.2

Difco™ Marine Broth 2216

Dehydrated Appearance: Light beige with a few dark particles, free flowing, homogeneous.

Solution: 3.74% solution, soluble in purified water upon boiling. Solution is light amber, slightly opalescent with precipitate.

Prepared Appearance: Light amber, slightly opalescent with a precipitate.

Reaction of 3.74%
Solution at 25°C: pH 7.6 ± 0.2

Cultural Response

Difco™ Marine Agar 2216

Prepare the medium per label directions. Inoculate and incubate at 20-25°C for 40-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Vibrio fischeri</i>	7744	10 ² -10 ³	Good
<i>Vibrio harveyi</i>	14126	10 ² -10 ³	Good

Difco™ Marine Broth 2216

Prepare the medium per label directions. Dispense 50 mL amounts in 250 mL Erlenmeyer flasks. Inoculate and incubate at 20-25°C on a shaker for 40-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Vibrio fischeri</i>	7744	10 ² -10 ³	Good
<i>Vibrio harveyi</i>	14126	10 ² -10 ³	Good

Uninoculated

The media contain minerals that nearly duplicate the major mineral composition of sea water,² in addition to peptone and yeast extract that provide a good source of nutrients.

In the use of Marine Agar 2216, the conventional pour plate and spread plate techniques of enumeration are used. For the pour plate technique, the agar must be cooled to 42°C before inoculation because of the thermo-sensitive nature of most marine bacteria. In the spread plate technique, the agar is poured while hot and allowed to cool and solidify before inoculation. This latter method was reported by Buck and Cleverdon³ to give higher counts than the pour plate method because of the increased growth of the thermo-sensitive bacteria.

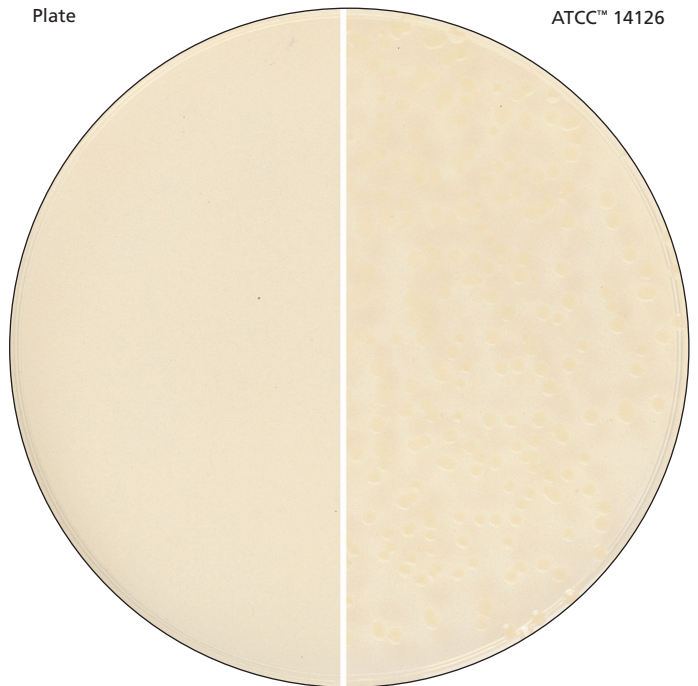
Sizemore and Stevenson⁴ used Marine Agar 2216 routinely as the upper nutrient layer of a marine agar-milk agar double-layer plate. This two layer plate was developed for isolating proteolytic marine bacteria. Marine Agar 2216 was also used in studies characterizing a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster).⁵

Principles of the Procedure

Peptone and yeast extract provide nitrogen, vitamins and minerals.

The high salt content helps to simulate sea water. Numerous minerals are also included to duplicate the major mineral composition of sea water. Agar is the solidifying agent.

Plate ATCC™ 14126



Formulae

Difco™ Marine Agar 2216

Approximate Formula* Per Liter	
Peptone	5.0 g
Yeast Extract	1.0 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride.....	8.8 g
Sodium Sulfate.....	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide.....	0.08 g
Strontium Chloride.....	34.0 mg
Boric Acid	22.0 mg
Sodium Silicate.....	4.0 mg
Sodium Fluoride	2.4 mg
Ammonium Nitrate	1.6 mg
Disodium Phosphate	8.0 mg
Agar	15.0 g

Difco™ Marine Broth 2216

Approximate Formula* Per Liter	
Peptone	5.0 g
Yeast Extract	1.0 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride.....	5.9 g
Magnesium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide.....	0.08 g
Strontium Chloride.....	34.0 mg
Boric Acid	22.0 mg
Sodium Silicate.....	4.0 mg
Sodium Fluoride	2.4 mg
Ammonium Nitrate	1.6 mg
Disodium Phosphate	8.0 mg

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
Difco™ Marine Agar 2216 - 55.1 g;
Difco™ Marine Broth 2216 - 37.4 g.
Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Consult appropriate references for recommended test procedures.^{3,4}

Expected Results

Refer to appropriate references and procedures for results.

References

1. ZoBell. 1941. J. Mar. Res. 4:42.
2. Lyman and Fleming. 1940. J. Mar. Res. 3:134.
3. Buck and Cleverdon. 1960. Limnol. Oceanogr. 5:78.
4. Sizemore and Stevenson. 1970. Appl. Microbiol. 20:991.
5. Weiner, Segall and Colwell. 1985. Appl. Environ. Microbiol. 49:83.

Availability

Difco™ Marine Agar 2216

Cat. No. 212185 Dehydrated – 500 g

Difco™ Marine Broth 2216

Cat. No. 279110 Dehydrated – 500 g
214907 Dehydrated – 10 kg