# 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 ZoBell[1] The media contain all of the nutrients necessary for the growth of marine bacteria. 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 Cleverdon[3] to give higher counts than the pour plate method because of the increased growth of the thermo-sensitive bacteria.

Sizemore and Stevenson[4] 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]

## 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 |
|---|---|---|---|
| *Vibrio fischeri* | 7744 | $10^2$-$10^3$ | Good |
| *Vibrio harveyi* | 14126 | $10^2$-$10^3$ | Good |

**Difco™ Marine Broth 2216**

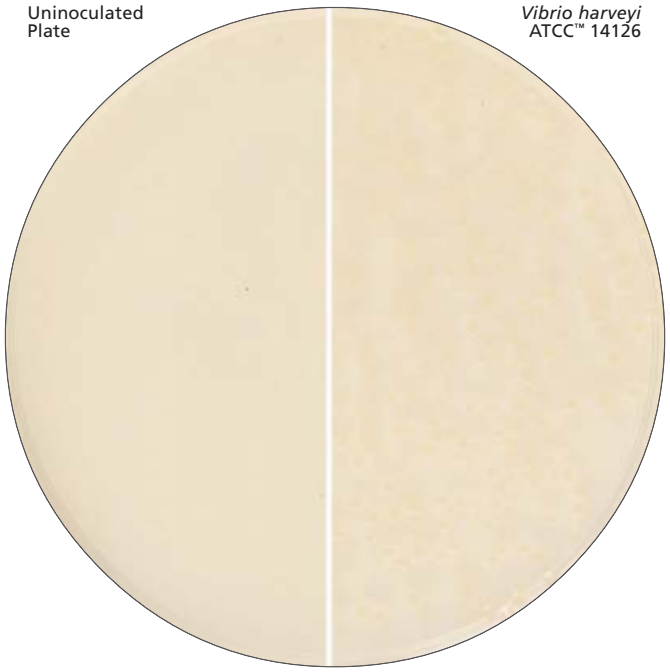
Prepare the medium per label directions. Dispense 50 mL amounts in 250 mL Erlenmeyer flasks. Inoculate and incubate at 20-25°C on a shaker for 40-72 hours.

| ORGANISM | ATCC™ | INOCULUM CFU | RECOVERY |
|---|---|---|---|
| *Vibrio fischeri* | 7744 | $10^2$-$10^3$ | Good |
| *Vibrio harveyi* | 14126 | $10^2$-$10^3$ | Good |

## 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.



## 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.
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

# Martin-Lewis Agar • Martin-Lewis Agar, Modified Martin-Lewis Agar (Gono-Pak) • Martin-Lewis Agar (JEMBEC™)

## Intended Use

Martin-Lewis Agar and Martin-Lewis Agar, Modified are used for the isolation of pathogenic *Neisseria* from specimens containing mixed flora of bacteria and fungi.

## Summary and Explanation

Thayer-Martin Selective Agar was developed for the primary isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens containing mixed flora taken from the throat, vagina, rectum, and urethra.[1-3] Consisting of **BBL**™ Chocolate II Agar with vancomycin, colistin and nystatin, it is formulated to minimize the overgrowth of gonococci and meningococci by contaminants, to suppress the growth of saprophytic *Neisseria* species and to enhance the growth of pathogenic *Neisseria*.

Martin et al. modified Thayer-Martin Selective Agar by adding trimethoprim to produce Modified Thayer-Martin (MTM) Selective Agar.[4] A significantly greater number of positive gonococcal isolates from clinical specimens was reported as compared with Thayer-Martin Selective Agar due to the inhibition of swarming *Proteus* species.[4-6] Because of its improved performance, it is recommended over earlier formulations for the isolation of *N. gonorrhoeae*.[7] The original formula contained 20 g/L of agar and 1.5 g/L dextrose (in addition to the dextrose in the **IsoVitaleX**™ Enrichment). The agar concentration has been changed to approximately 12 g/L; the extra 1.5 g/L of dextrose has been eliminated since the lower dextrose content was found to improve the growth of *N. gonorrhoeae*. **BBL** MTM II was developed by careful selection and pretesting of raw materials to provide enhanced growth of gonococci as well as improved inhibition of *Candida* species.

Also recommended over earlier formulations is Martin-Lewis Agar, a further modification of the earlier formulations