M-BROTH (7296)

Intended Use
M-Broth is used for the cultivation of *Salmonella* spp.

Product Summary and Explanation
M-Broth is prepared according to the formula of Sperber and Diebel,[1] and contains the nutrients necessary for good growth and flagella development of *Salmonella*. M-Broth is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure. M-Broth conforms to standard procedures recommended in food testing and monoclonal and polyclonal enzyme immunoassays (EIA).[2,3]

Principles of the Procedure
Enzymatic Digest of Casein is the nitrogen source in M-Broth. Yeast Extract is a source of B-complex vitamins. D-Mannose and Sodium Citrate are the fermentation energy sources. Mannose prevents fimbrial agglutination.[1] Sodium Chloride helps maintain osmotic equilibrium, while Dipotassium Phosphate acts as a buffer. The inorganic salts stimulate bacterial growth. Polysorbate 80 is a surfactant and dispersing agent.

Formula / Liter
Enzymatic Digest of Casein ..... 12.5 g
Yeast Extract ..... 5 g
D-Mannose ..... 2 g
Sodium Citrate ..... 5 g
Sodium Chloride ..... 5 g
Potassium Phosphate ..... 5 g
Manganese Chloride ..... 0.14 g
Magnesium Sulfate ..... 0.8 g
Ferrous Sulfate ..... 0.04 g
Polysorbate 80 ..... 0.75 g
Final pH: 7.0 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Dissolve 36.2 g of the medium in one liter of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous with small lumps and light beige.

Prepared Appearance: Prepared medium is yellow to amber and clear to trace hazy.

Expected Cultural Response: Cultural response in M-Broth at 35 ± 2°C and examined for growth after 18 - 24 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Salmonella arizonae ATCC® 13314	10 - 300	Good growth
Salmonella choleraesuis ATCC® 13076	10 - 300	Good growth
Salmonella typhimurium ATCC® 14028	10 - 300	Good growth
Salmonella typhi ATCC® 19430	10 - 300	Good growth

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at 35 ± 2°C for 18 - 24 hours.
2. Transfer 1 mL of the above pre-enrichment culture to 9 mL of Selenite Cystine Broth, and 1 mL to 9 mL of Tetrathionate Broth. Incubate both enrichment media at 35 ± 2°C for 24 hours.
3. Inoculate one 10 mL tube of M-Broth, tempered to 35°C, with one drop from each of the above cultures. Incubate at 35 ± 2°C for 6 - 8 hours.
4. Prepare a formalin-salt solution by adding 4.2 grams of NaCl and 3 mL of formalin to 100 mL of distilled water. Place one drop in each of two Kahn tubes.
5. Carefully insert a pipette about 1 inch below the surface of the M-Broth culture and transfer 0.85 mL of culture to each of the above Kahn tubes containing formalin-salt solution.
6. Prepare a pooled antiserum by combining together 0.5 mL each of rehydrated Salmonella H Antiserum Poly D and Salmonella H Antiserum z_6 in 11.5 mL of 0.85% NaCl.
7. Add 0.1 mL pooled Salmonella H Antiserum to one of the Kahn tubes (above). Add 0.1 mL of 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C water bath for 1 ½ hours.

Note: An alternative testing procedure can be found in AOAC International[3] for screening procedures using enzyme immunoassay or DNA hybridization to detect Salmonella antigens in test samples.

Results

Agglutination in the Kahn tube containing salmonella antiserum indicates the presence of *Salmonella*. agglutination in the Kahn tube containing 0.8% NaCl solution (control tube) indicates a rough culture which should be streaked for isolation, passed through Motility GI Medium to enhance flagella, and then retested with pooled antiserum.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Packaging

M-Broth	**Code No.**	**7296A**	**500 g**
		7296B	**2 kg**
		7296C	**10 kg**

References

1. **Sperber, W. H., and R. H. Deibel.** 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl Microbiol. **17:**533-539.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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