TETRATHIONATE BROTH BASE, HAJNA (7740)

Intended Use
Tetrathionate Broth Base, Hajna is used as a supplement with for the isolation of Salmonella species (except S. typhi) from samples in a laboratory setting. Tetrathionate Broth Base, Hajna is not intended for use in the diagnosis of disease or other conditions in humans.

Product Summary and Explanation
Tetrathionate Broth Base, Hajna is used as a selective enrichment for the cultivation of Salmonella spp. Salmonella organisms may be present but injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservative, and sanitizers.1 From May 1 to November 30, 2010, over 19000 reported cases of Salmonella Enteritidis (SE) infections were associated with contaminated shell eggs.2 SE infections can be very serious and symptoms can included diarrhea, fever, abdominal cramps, headache, nausea and vomiting.3 Eggs can become contaminated on the farm because a laying hen can become infected with SE and pass the bacteria into the egg before it is laid.3 If the egg is not refrigerated, the bacteria can grow inside the uncracked, whole egg.3 Tetrathionate Broth Base, Hajna, is recommended in the FDA/BAM Method for the Environmental Sampling and Detection of Salmonella Enteritidis (SE) in Poultry Houses4 and the Compendium of Methods for the Microbiological Examination of Foods.5 Tetrathionate Broth Base, Hajna was developed by Hajna and Damon.6

Principles of the Procedure
Casein/Meat Peptone provides nitrogen, carbon, vitamins, and amino acids in Tetrathionate Broth Base, Hajna. Dextrose and Mannitol are the fermentable carbohydrates. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.7 Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will flourish in this medium.8 Bile Salts, a selective agent, suppresses coliform bacteria and inhibits Gram-positive organisms. Calcium Carbonate neutralizes and absorbs toxic metabolites.

Formula / Liter

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Composition per 40.0 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Thiosulfate</td>
<td>38 g</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>25 g</td>
</tr>
<tr>
<td>Casein/Meat Peptone (50:50)</td>
<td>18 g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5 g</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>2.5 g</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>2 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Sodium Deoxycholate</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Brilliant Green</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Final pH:</td>
<td>7.6 ± 0.2 at 25°C</td>
</tr>
</tbody>
</table>

Formula may be adjusted and/or supplemented as required to meet performance specifications.

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

Directions
1. Suspend 91.5 grams of the medium in 960 milliliters of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE. Cool to 40 - 45°C.
4. Just prior to inoculation, add 40 mL of Iodine/Potassium Iodide Solution to the prepared Tetrathionate Broth Base, Hajna.
5. Distribute into sterile capped containers in 10 ml volumes.
   Note: Do not add Iodine/Potassium Iodide Solution to tubes until just before inoculation. Chemical Tetrathionate inhibits by oxidation of Thiosulfate through the addition of Iodine just prior to use.8
Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light mint green.

Prepared Appearance: Prepared medium is light green, opaque to opalescent with a heavy precipitate.

Expected Cultural Response: Cultural response after aerobic incubation in supplemented Tetrathionate Broth Base, Hajna, for 18 – 24 hours at 35 ± 2°C. Inoculated Tetrathionate Broth Base, Hajna was subcultured onto MacConkey Agar and examined for recovery after incubation at 35 ± 2°C for 18 – 24 hours.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Approx. Inoculum (CFU)</th>
<th>Expected Results (MacConkey Agar)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC® 25922</td>
<td>300 - 1000</td>
<td>None to poor recovery; pink with bile precipitate where recovered.</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em> ATCC® 14028</td>
<td>300 - 1000</td>
<td>Good growth; colorless colonies</td>
</tr>
</tbody>
</table>

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

Test Procedure
For a complete discussion of the isolation and identification of *Salmonella*, refer to appropriate references.

For the Environmental Sampling and Detection of *Salmonella* Enteritidis in Poultry Houses, refer to FDA, U.S. Food and Drug Administration.

Results
Positive – Growth, indicated by turbidity.
Negative – No growth, medium remains clear.

Storage
Store sealed bottle containing the dehydrated medium at 2 - 30°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.

Limitations of the Procedure
1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. Do not add Iodine-Potassium Iodide Solution to tubes until just before inoculation. Chemical Tetrathionate inhibits by oxidation of Thiosulfate through addition of Iodine just prior to use.
3. Do not attempt to get Tetrathionate Broth Base, Hajna completely into solution on heating, Calcium carbonate is present in a supersaturated concentration. Mix well, swirl flask while dispensing medium to keep ingredients in a uniform suspension.

Packaging
Tetrathionate Broth Base, Hajna

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>7740A</td>
<td>500 g</td>
</tr>
<tr>
<td>7740B</td>
<td>2 kg</td>
</tr>
<tr>
<td>7740C</td>
<td>10 kg</td>
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</tbody>
</table>
References
2. www.cdc.gov/salmonella/enteritidis/

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.