



## TETRATHIONATE BROTH BASE (7241)

### Intended Use

**Tetrathionate Broth Base** is used with iodine for the recovery of *Salmonella* spp.

### Product Summary and Explanation

Tetrathionate Broth Base is used as a selective enrichment for the cultivation of *Salmonella* spp. that may be present in small numbers and compete with intestinal flora. *Salmonella* organisms may also be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservative, and sanitizers.<sup>1</sup> *Salmonella* spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.<sup>2</sup>

Mueller<sup>3</sup> demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms. Using modified Mueller's broth, Kauffmann<sup>4,5</sup> increased the number of positive isolates. Tetrathionate Broth was used in studies for the poultry industry<sup>6,7</sup> and in a collaborative study for rapid screening of *Salmonella* in food.<sup>8</sup> Tetrathionate Broth Base, abbreviated as TT Broth Base, is specified in standard methods<sup>9-12</sup> for *Salmonella* testing. The FDA, Bacteriological Analytical Manual incorporate Tetrathionate Broth Base as a pre-enrichment medium for detecting *Salmonella* in food materials.<sup>13</sup> Tetrathionate Broth Base is used in processing fecal cultures for bacteria.<sup>14</sup>

### Principles of the Procedure

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provides nitrogen, carbon, vitamins, and amino acids in Tetrathionate Broth Base. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.<sup>15</sup> Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Bile Salts, a selective agent, suppresses coliform bacteria and inhibits Gram-positive organisms. Calcium Carbonate neutralizes and absorbs toxic metabolites.

### Formula / Liter

Enzymatic Digest of Casein .....	2.5 g
Enzymatic Digest of Animal Tissue.....	2.5 g
Bile Salts .....	1 g
Calcium Carbonate .....	10 g
Sodium Thiosulfate .....	30 g

Final pH: 8.4 ± 0.2 at 25°C

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

### Supplement

Iodine-Potassium Iodide Solution	
Composition per 20.0 mL	
KI	5.0 g
Iodine	6.0 g

### Precautions

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

### Directions

1. Dissolve 46 g of the medium in one liter of purified water.
2. Heat with frequent agitation to boiling.
3. Cool to 45°C and add 20 mL of the Iodine-Potassium Iodide Solution to the prepared Tetrathionate Broth Base. If preparing solution, add 6 grams Iodine + 5 grams Potassium Iodide in 20 mL of purified water.
4. DO NOT REHEAT AFTER ADDING IODINE SOLUTION.

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.<sup>16</sup>

### Quality Control Specifications

**Dehydrated Appearance:** Powder is homogeneous, free flowing, and white to off-white.

**Prepared Appearance:** Prepared medium is milky white to slightly yellow-white and opaque.

**Expected Cultural Response:** Cultural response after enrichment in Tetrathionate Broth Base (with the Iodine/Iodide solution) and subcultured to MacConkey Agar. Cultures were incubated aerobically at  $35 \pm 2^\circ\text{C}$  and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
<i>Escherichia coli</i> ATCC® 25922	~ 1000	Inhibited
<i>Salmonella arizonae</i> ATCC® 13314	10 - 300	Growth
<i>Salmonella typhimurium</i> ATCC® 14028	10 - 300	Growth
<i>Shigella flexneri</i> ATCC® 12022	~ 1000	Inhibited

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.

### **Results**

Refer to appropriate references for results.

### **Storage**

Store sealed bottle containing the dehydrated medium at  $2 - 30^\circ\text{C}$ . 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 grow poorly or fail to grow on this medium.

### **Packaging**

<b>Tetrathionate Broth Base</b>	<b>Code No.</b>	<b>7241A</b>	<b>500 g</b>
		<b>7241B</b>	<b>2 kg</b>
		<b>7241C</b>	<b>10 kg</b>

### **References**

- Hartman, P. A., and S. A. Minnich. 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
- Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.). 1995. Manual of clinical microbiology, 6<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
- Mueller, L. 1923. Un Nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C. R. Soc. Bio. **89**:434. Paris.
- Kauffmann, F. 1930. Ein kombiniertes anreicherungsverfahren fur typhus und-paratyphusbacillen. Zentralb. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I orig. **113**:148.
- Kauffmann, F. 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren fur Salmonella bacillen. Z. Hyg. Infektionskr. **117**:26.
- Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. Tarver, Jr., R. L. Walker, and M. J. Wineland. 1991. A survey of *Salmonella* contamination in modern broiler production. J. Food Prot. **54**:502-507.
- Barnhart, H. M., D. W. Dressen, R. Bastien, and O. C. Pancorbo. 1991. Prevalence of *Salmonella enteritidis* and other serovars in ovaries of layer hens at time of slaughter. J. Food Prot. **54**:488-492.
- Eckner, K. F., W. A. Dustman, M. S. Curiale, R. S. Flowers, and B. J. Robison. 1994. Elevated-temperature, colorimetric, monoclonal, enzyme linked immunosorbent assay for rapid screening of *Salmonella* in foods; collaborative study. J. Assoc. Off. Anal Chem. **77**:374-383.

9. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
10. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
11. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23<sup>rd</sup> ed. The United States Pharmacopeial Convention. Rockville, MD.
12. **Federal Register.** 1991. Animal and plant health inspection service: chicken affected by *Salmonella enteritidis*, final rule, Fed. Regist. **56**:3730-3743.
13. **[www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm).**
14. **Isenberg, H. D. (ed.).** 1992 Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology. Washington, D. C.
15. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol. **54**:469-483.
16. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

### **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.