



## BD™ Tetrathionate Broth Base • BD Iodine Solution (for Tetrathionate Broth Base)

### INTENDED USE

**BD Tetrathionate Broth Base**, after supplementation with **BD Iodine Solution**, is a selective enrichment medium for *Salmonella* species from human stools and from a variety of foods. The completed medium is also known as TT Broth.

### PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

*Salmonella* species cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.<sup>1</sup> The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than two days and diarrhea lasting less than 7 days.<sup>1</sup> Mueller demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms.<sup>2</sup> Using modified Mueller's broth, Kauffmann increased the number of positive isolates.<sup>3,4</sup> Tetrathionate Broth was used in studies for the poultry industry and in a collaborative study for rapid screening of *Salmonella* in food.<sup>5-7</sup> Tetrathionate Broth is specified in standard methods for *Salmonella* testing and is used in processing fecal cultures for bacteria.<sup>8-15</sup>

Tetrathionate Broth Base, when supplemented with iodine/iodide solution, is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in small numbers and compete with intestinal flora. As a result, they may not be detected by primary plating of the specimen on selective differential solid media.

In **BD Tetrathionate Broth Base**, Proteose Peptone provides nitrogen, carbon, vitamins and amino acids. Selectivity is accomplished by the combination of sodium thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.<sup>16</sup> (Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide contained in **BD Iodine Solution**.) Organisms containing the enzyme tetrathionate reductase will proliferate in the medium.<sup>17</sup> Bile salts, a selective agent, suppress coliform bacteria and inhibit gram-positive organisms. Calcium carbonate neutralizes and absorbs toxic metabolites and provides a stable pH value.

### REAGENTS

Formulas\* Per Liter Purified Water

BD Tetrathionate Broth Base		BD Iodine Solution	
<b>Bacto™</b> Proteose Peptone	5.0 g	Iodine	300.0 g
<b>Bacto</b> Bile Salts	1.0	Potassium Iodide	250.0
Sodium Thiosulfate	30.0	Appearance: Reddish-brown	
Calcium Carbonate	10.0		
pH 8.4 +/- 0.2			
Appearance: Nearly colorless to slightly yellowish, with heavy white precipitate			
Appearance after addition of iodine solution: Brownish, heavy precipitate			

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

### PRECAUTIONS

**IVD** . For professional use only.

Do not use vials or bottles if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration. For completion of **BD Tetrathionate Broth Base**, follow the procedures described under **Reagent Preparation**.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

**Warning: BD Iodine Solution (for Tetrathionate Broth Base): Hazard-determining components of labeling: iodine**



**Xn Harmful**

**Risk phrases:**

20/21 Harmful by inhalation and in contact with skin.

**Safety phrases:**

9 Keep container in a well-ventilated place.

23 Do not breathe fumes/vapour.

25 Avoid contact with eyes.

36/37 Wear suitable protective clothing and gloves.



**N Dangerous for the environment**

**Risk phrases:**

50 Very toxic to aquatic organisms.

**Safety phrases:**

57 Use appropriate container to avoid environmental contamination.

**STORAGE AND SHELF LIFE**

On receipt, store vials with **BD Tetrathionate Broth Base** in the dark at 5 to 25° C until just prior to use. Avoid freezing and overheating. The vials may be inoculated up to the expiration date and incubated for the recommended incubation times. Vials from opened packages can be used up to the expiration date. Opened vials must be used immediately.

Store **BD Iodine Solution** at 15 to 22° C in the dark; do not refrigerate. Close the bottle tightly! Bottles from opened packages can be used up to the expiration date. Opened bottles can be used repeatedly up to the expiry date if closed and stored properly after each use.

**USER QUALITY CONTROL**

Before use, complete **BD Tetrathionate Broth Base** by adding **BD Iodine Solution** (for details, see **PROCEDURE - Reagent Preparation**). Inoculate the vials with 10 to 100 CFU of the *Salmonella* strains per vial. Use 10<sup>4</sup> to 10<sup>5</sup> CFU for the remaining strains. Incubate for 18 to 24 h at 35 ± 2° C. After incubation, subculture 10 to 20 µl onto **BD MacConkey II Agar** plates; incubate the plates for 18 to 24 hours at 35 ± 2° C and record growth.

Strains	Growth	Growth upon subculture on BD MacConkey II Agar
<i>Salmonella</i> Typhimurium ATCC™ 14028	Good to excellent	Good to excellent
<i>Salmonella</i> Abony DSM 4224	Good to excellent	Good to excellent
<i>Escherichia coli</i> ATCC 25922	Weak to good	Inhibition partial
<i>Enterococcus faecalis</i> ATCC 19433	Weak or none	Inhibition partial to complete
<i>Staphylococcus aureus</i> ATCC 25923	Weak or none	Inhibition partial to complete

**PROCEDURE**

**Materials Provided**

**BD Tetrathionate Broth Base**, 12 ml, provided in 30 ml screw-cap vials. Microbiologically controlled.

**BD Iodine Solution (for Tetrathionate Broth Base)**, 40 ml, provided in 50 ml bottles with screw cap.

**Materials Not Provided**

Ancillary culture media, reagents and laboratory equipment as required.

### Reagent Preparation

To complete **BD Tetrathionate Broth Base**, add 2% (or 0.24 ml) of **BD Iodine Solution (for Tetrathionate Broth Base)** per vial (=12 ml). After completion, close the tube and mix gently. Do not heat! Note that the white sediment (calcium carbonate) does not dissolve after completion. Inoculate the completed medium within 2 hours after addition of the iodine solution!

### Specimen Types

The completed Tetrathionate Broth is used as an enrichment broth for *Salmonella* from a variety of foods and from human stool specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

### Test Procedure

Add 1.0 to 3.0 g of solid specimens or 1.0 to 3.0 ml of liquid specimens per tube of completed Tetrathionate Broth. Smaller amounts of stool may be used during the acute phase of an infection. Mix carefully and incubate for 12 to 24 hours at  $35 \pm 2^\circ \text{C}$  in an aerobic atmosphere. Food materials with high numbers of contaminants are incubated at  $43 \pm 0.2^\circ \text{C}$  in a waterbath. For a complete discussion of the appropriate procedures for foods, refer to the references.<sup>8-12</sup> Note that stool specimens should also be streaked directly (without prior enrichment) onto appropriate plated media.<sup>1,14,15</sup>

### Results

After the incubation, subculture Tetrathionate medium onto appropriate selective differential media, e.g. **BD XLD Agar** or **BD Hektoen Enteric Agar** and on **BD MacConkey II Agar**. Consult the references.<sup>1,11-15</sup>

### PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

**BD Tetrathionate Broth Base**, after completion with **BD Iodine Solution**, is a standard medium which is used for the enrichment of *Salmonella* species from foods and human stool specimens.<sup>1,8-15</sup> Tetrathionate broth and Rappaport-Vassiliadis medium were shown to be the most sensitive media for food materials with low *Salmonella* counts.<sup>18</sup>

Since the nutritional requirements of organisms vary, some strains of *Salmonella* may be encountered that fail to grow or grow poorly in this medium. Isolation techniques should always include a variety of enrichment broths and isolation media. Consult the references.<sup>1,14,15</sup>

Isolates obtained from this medium must be subjected to further biochemical and serological tests to obtain a complete identification.<sup>1,14,15</sup>

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## PACKAGING/AVAILABILITY

### **BD Tetrathionate Broth Base** (partially completed bottled medium)

Cat. No. 257103                      cpu 50                      12 ml fill volume; in 30 ml screw-cap vials

### **BD Iodine Solution (for Tetrathionate Broth Base)** (partially completed bottled medium)

Cat. No. 257199                      cpu 1:                      40 ml fill volume; in a 50 ml screw cap bottle

## FURTHER INFORMATION

For further information please contact your local BD representative.



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