



## PRODUCT SPECIFICATION SHEET

### Tetrathionate Broth Base, Hajna (TT Broth Base) (DM260)

#### Intended Use

Tetrathionate Broth Base, Hajna (TT Broth Base) (DM260) is recommended for enrichment and isolation of *Salmonellae*.

#### Product Summary and Explanation

*Salmonella* is the common causative agent of mild gastroenteritis to typhoid.<sup>(1)</sup> The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.<sup>(2)</sup> It is common contaminant in food and other biological products. *Salmonella* organisms can be injured in food-processing procedures. These procedures include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers. Although injured cells may not form colonies on selective media, they can cause disease if ingested.<sup>(3)</sup> Tetrathionate Broth Base was first formulated by Mueller<sup>(4)</sup> who showed that this medium favours the unrestricted growth of enteric pathogens by selectively inhibiting the coliforms. Muellers medium was subsequently modified by Kauffman<sup>(5)</sup> and Knox<sup>(6)</sup> in which they obtained more number of isolates. Tetrathionate Broth Base, Hajna is the modification formulated by Hajna and Damon.<sup>(7)</sup> Hajna and Damon developed a new broth containing yeast extract, peptone, carbon sources and the selective agents, sodium desoxycholate and brilliant green (replacing bile salts). TT Broth Base, Hajna is used in testing *Salmonella* in egg processing plants. It is included in procedures for the isolation and identification of *Salmonella* from meat and poultry as well as egg products. This medium is recommended by APHA<sup>(8)</sup> for the selective enrichment of Salmonellae from foodstuffs.

#### Principles of the Procedure

Tetrathionate Broth Base, Hajna contains peptone special which are the sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies growth factors and vitamins. Dextrose and mannitol are fermentable carbohydrates. The selectivity depends on the ability of thiosulphate and tetrathionate to suppress commensal coliform organisms. Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide.<sup>(9,10)</sup> Sodium deoxycholate and brilliant green inhibit gram-positive organisms. Calcium carbonate neutralizes the acidic tetrathionate decomposition products. Sodium chloride maintains the osmotic balance of the medium.

#### Formula / Liter

Ingredients	Gms / Liter
Peptone, special	18.00
Yeast extract	2.00
Sodium chloride	5.00
D-Mannitol	2.50
Dextrose	0.50
Sodium deoxycholate	0.50
Sodium thiosulphate	38.00
Calcium carbonate	25.00
Brilliant green	0.01
Final pH: 7.6 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	





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### 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 one liter of distilled water.
2. Heat just to boiling or place in flowing steam for 30 minutes.
3. DO NOT AUTOCLAVE. Cool to 45°C.
4. Mix and add 40 ml of Iodine solution (8 g potassium iodide and 5 g iodine per 40 ml).
5. Mix and dispense 10 ml amounts in tubes.
6. Do not heat after addition of iodine.

### Quality Control Specifications

Dehydrated Appearance	Cream to light green homogeneous free flowing powder
Prepared Medium	Light green coloured opalescent solution with white precipitate, on standing the precipitate settles down
Reaction of 9.15% Solution	pH : 7.6 ± 0.2 at 25°C
Gel Strength	Not Applicable

**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours (Recovery is done on MacConkey Agar DM143).

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth on MacConkey Agar	Colour of colony
1.	<i>Escherichia coli</i> ATCC 25922	50 -100	fair to good	pink-red with bile precipitate
2.	<i>Salmonella Arizonae</i> ATCC 13314	50 -100	good-luxuriant	colourless
3.	<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	good-luxuriant	colourless
4.	<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	good-luxuriant	colourless

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

### Test Procedure

1. After preparation, add 1-3 g of fecal specimen to each tube (heavy inoculum).
2. Incubate tubes for 12-24 hours at 35 ± 2°C in an aerobic atmosphere. Refer to appropriate references for standard test procedures.

### Results

1. Growth is indicated by turbidity in the medium. Subculture to selective and differential enteric plating media like Brilliant Green Agar (DM044), MacConkey Agar (DM143), Bismuth Sulphite Agar (DM039) for further confirmation, for further investigations.
2. Refer to appropriate references and standard procedures for interpretation of results.





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### Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### Expiration

Refer to the 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 the presence of calcium carbonate, the prepared medium forms opalescent solution with a white precipitate.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

**Product Name :** Tetrathionate Broth Base, Hajna (TT Broth Base)

**Product Code :** DM260

**Available Pack sizes :** 100gm / 500gm

### References

1. Hartman and Minnich. 1981. J. Food Prot. 44:385.
2. Gray. 1995. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 6<sup>th</sup> ed. American Society for Microbiology, Washington, D.C.
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4. Mueller L., 1923, C.R. Soc. Biol. (Paris), 89:434.
5. Kauffman F., 1930, Zentralb. Bakteriol. Parasitenkd. Infektionskr.-Hyg. Abt. I. Orig., 113:148.
6. Knox R., Gell P. and Pollack M., 1942, J. Pathol. Bacteriol, 54:469.
7. Hajna A. A. and Damon S. R., 1956, Appl. Microbiol., 4:341.
8. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4<sup>th</sup> Ed., APHA, Washington, D.C.
9. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
10. Pollock M. R. and Knor R., 1943, Biochem J., 37:476.

### Further Information

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