Record ID: FS-TSS-0291 Revision Number: 3.0 Effective Date: 2023-08-14 12:00 AM EDT

Tetrathionate (TT) Broth Base, Hajna
SKU: 700003380, 700003381, 700003382, 700003383
NCM0143

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

Description
Tetrathionate Broth Base, Hajna, is recommended in the Compendium of Methods for the Microbiological Examination of Foods and the FDA/BAM for the Environmental Sampling and Detection of Salmonella Enteritidis (SE) in Poultry Houses and whole eggs.. *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. Numerous reported cases of *Salmonella enteritidis* (SE) infections are associated with contaminated shell eggs. SE infections can be very serious and symptoms can include diarrhea, fever, abdominal cramps, headache, nausea and vomiting. 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. If the egg is not refrigerated, the bacteria can grow inside the uncracked, whole egg. Tetrathionate Broth Base, Hajna was developed by Hajna and Damon.

Typical Formulation

Sodium Thiosulfate	38.0 g/L
Calcium Carbonate	25.0 g/L
Peptone Mixture	18.0 g/L
Sodium Chloride	5.0 g/L
D-Mannitol	2.5 g/L
Yeast Extract	2.0 g/L
Glucose	0.5 g/L
Sodium Deoxycholate	0.5 g/L
Brilliant Green	0.01 g/L

pH: 7.6 ± 0.2 at 25°C
Formula is adjusted and/or supplemented as required to meet performance specifications.

Supplement/Liter
Iodine-Potassium Iodide Solution
Composition per 40 mL

KI	8 g/L
Iodine	5 g/L

Precaution
Refer to SDS

Preparation
1. Suspend 91.5g of the medium in 960 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE. Cool to 45 - 50°C.
4. Just prior to inoculation, add 40 mL of Iodine/Potassium Iodide Solution to the prepared Tetrathionate Broth Base, Hajna
5. Dispense into sterile capped containers in 10 mL volumes.

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Note: Do not add Iodine/Potassium Iodide Solution to tubes until just before inoculation. Tetrathionate inhibits by oxidation of Thiosulfate through the addition of Iodine just prior to use.

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.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and off-white to beige to 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 (TT) Broth Base, Hajna, for 18 – 24 hours at 33-38°C. Inoculated Tetrathionate (TT) Broth Base, Hajna was sub-cultured onto XLD Agar (NCM0021) and TSA (NCM0002) and examined for recovery after incubation at 33-38°C for 18 – 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results
Escherichia coli ATCC® 25922	>1000	TSA- None to poor recovery
Enterococcus faecalis ATCC® 29212	>1000	TSA- Satisfactory inhibition
Salmonella typhimurium ATCC® 14028	10-100	XLD- Good growth; red w/ black center colonies
Salmonella enterica ATCC® 13076	10-100	XLD- Good growth; yellow to red w/ black center colonies
Pseudomonas aeruginosa ATCC® 27853	>1000	XLD- Satisfactory suppression

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

Results

Degradation of xylose, lactose, and sucrose generates acid products, causing a color change in the colonies and in the medium from red to yellow.

Hydrogen sulfide production under alkaline conditions results in colonies with black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.

Lysine decarboxylation, in the absence of lactose and sucrose fermentation, results in a reversion to an alkaline condition. This alkaline condition causes the color of the medium to change back to red.

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.

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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 (TT) 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.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. 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.

References

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