



## PRODUCT SPECIFICATION SHEET

### Broth Medium I (Tetrathionate Bile-Brilliant Green Broth) (DM712E)

#### Intended Use

Broth Medium I (Tetrathionate Bile-Brilliant Green Broth) (DM712E) is recommended for isolation and identification of *Salmonellae*, in compliance with EP

#### Product Summary and Explanation

*Salmonella* are gram-negative, facultatively anaerobic, non-sporulating, non-motile rods in the family *Enterobacteriaceae*. They are widely distributed in animals affecting mainly the stomach and the intestines. These organisms are difficult to differentiate biochemically from *Escherichia coli*. Mueller<sup>(1)</sup> first described Tetrathionate Broth, which was later modified by Kauffman.<sup>(2, 3)</sup> Tetrathionate Brilliant Green Bile Broth is used as an enrichment medium for *Salmonella*. Enrichment broth is usually recommended to facilitate the recovery of small numbers of *Salmonella* species.<sup>(4)</sup> Tetrathionate Bile Brilliant Green Broth cited as Broth Medium I is prepared as per the recommendation of European Pharmacopoeia<sup>(5)</sup> and is used for isolation and identification of *Salmonella* species in the tests prescribed for sterility checking in the Pharmacopoeia. It is also used to detect *Salmonella* from pharmaceutical, foods, water and other materials of sanitary importance.

#### Principles of the Procedure

Broth Medium I (Tetrathionate Bile-Brilliant Green Broth) contains peptone which provides nitrogenous nutrients for growth of *Salmonellae*. Brilliant green and ox-bile inhibit both gram-positive as well as some selected gram-negative organisms. They also prevent the growth of the anaerobic lactose fermenters such as *Clostridium perfringens*, which could give false positive reactions at 44°C. Potassium tetrathionate inhibits normal flora of faecal specimens. Organisms that have the enzyme tetrathionate reductase will grow and multiply in this medium due to the presence of Potassium tetrathionate. Calcium carbonate buffers sulfuric acid produced on reduction of tetrathionate. Sodium chloride helps in maintaining osmotic equilibrium.

#### Formula / Liter

Ingredients	Gms / Liter
Peptone	8.60
Ox bile	8.00
Sodium chloride	6.40
Calcium carbonate	20.00
Potassium tetrathionate	20.00
Brilliant green	0.07
Final pH: 7.0 ± 0.2 at 25°C	
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.
3. Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with white precipitate.





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### Directions

1. Suspend 63.07 grams of the medium in one liter of distilled water.
2. Heat just to boiling. Mix well and distribute into final containers.
3. DO NOT AUTOCLAVE OR REHEAT.

### Quality Control Specifications

<b>Dehydrated Appearance</b>	Light yellow to pale green homogeneous free flowing powder
<b>Prepared Medium</b>	Bluish green coloured opalescent solution with white precipitate.
<b>Reaction of % Solution</b>	Not Applicable
<b>Gel Strength</b>	Not Applicable

**Expected Cultural Response:** Cultural characteristics observed after enrichment in Broth Medium I at 41-43°C for 18-24 hours, and then subcultured on Agar Medium K (DM297E) and Agar Medium L (DM044E) and incubated at 35-37°C for specified period.

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Observed Lot Value (CFU)	Recovery	Colour of colony	Incubation Period
<b>Growth on Agar Medium K</b>							
1.	<i>Salmonella Typhimurium</i> ATCC 14028	50 -100	luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
2.	<i>Salmonella Abony</i> NCTC 6017	50 -100	good-luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
<b>Additional Microbiological Testing Growth on Agar Medium K</b>							
3.	<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	luxuriant	25 -100	≥50 %	red with black centres	18 -72 hrs
4.	<i>Staphylococcus aureus</i> ATCC 6538	≥10 <sup>3</sup>	inhibited	0	0%	--	≥72 hrs
5.	<i>Escherichia coli</i> ATCC 8739	50-100	fair	10 -30	20 -30 %	yellow	18 -72 hrs
<b>Growth on Agar Medium L</b>							
6.	<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	25 -100	≥50 %	pinkish white	18 -72 hrs
7.	<i>Salmonella Abony</i> NCTC 6017	50-100	luxuriant	25 -100	≥50 %	pinkish white	18 -72 hrs
<b>Additional Microbiological Testing Growth on Agar Medium L</b>							
8.	<i>Salmonella Enteritidis</i> ATCC 13076	50-100	luxuriant	25 -100	≥50 %	pinkish white	18 -72 hrs





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9.	<i>Staphylococcus aureus</i> ATCC 6538	$\geq 10^3$	inhibited	0	0%	--	$\geq 72$ hrs
10.	<i>Escherichia coli</i> ATCC 8739	50-100	fair	10 -30	20 -30 %	yellow	18 -72 hrs

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

### Test Procedure

1. Enrich the sample in a suitable enrichment medium.
2. Inoculate the pre-enriched culture in an appropriate amount of Broth Medium I and incubate for 18-24 hours at 35-37°C. (selective enrichment).
3. Further streak onto differential medium for isolation and identification.

### Results

Refer appropriate references and test procedures for interpretation of results.

### 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. Tetrathionate Brilliant Green Bile Broth is not suitable for growth of *Salmonella typhi* and *Salmonella paratyphi*.
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 : Broth Medium I (Tetrathionate Bile-Brilliant Green Broth)

Product Code : DM712E

Available Pack sizes : 500gm

### References

1. Mueller L., 1923, C. R. Soc. Biol., (Paris), 89, 434.
2. Kauffman F., 1930, Hyg. Abt. I. Orig., 113, 148.
3. Kauffman F., 1935, Z. Hyg. Infektionskr., 117, 26.
4. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Ed.). 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
5. European Pharmacopoeia, 2008, European Dept. for the Quality of Medicines.





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### Further Information

For further information please contact your local MICROMASTER Representative.



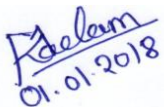


**MICROMASTER LABORATORIES PRIVATE LIMITED**

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Unit 38/39, Kalpataru Industrial Estate,  
Near Runwal Estate, Behind 'R-Mall' ,Ghodbunder Raod,  
Thane (W) - 400607. M.S. INDIA.

Ph: +91-22-25895505, 4760, Cell: 9320126789.

Email: [micromaster@micromasterlab.com](mailto:micromaster@micromasterlab.com)  
[sales@micromasterlab.com](mailto:sales@micromasterlab.com)

Prepared By	Checked By	Approved By
 01.01.2018	 01.01.2018	 01.01.2018
Microbiologist	Head Quality Control	Head Quality Assurance

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