

## TRIPLE SUGAR IRON AGAR (TSI)

AGTS-00I-500

- **Principle**

Triple Sugar Iron Agar (TSI) is recommended by ISO 6579 and ISO 19250 for the biochemical confirmation of Salmonella.

Peptone and the beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. The three carbohydrates (glucose, sucrose and lactose) are the fermentable carbohydrates providing carbon and energy. When these are fermented the acid production is indicated by the phenol red indicator, being the colour changes yellow for acid production and red for alkalization. Sodium thiosulfate is reduced to hydrogen sulphide, which reacts with the iron salt to give the black iron sulphide. The ferric ammonium citrate is a H<sub>2</sub>S indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

The glucose concentration in the medium is one-tenth the concentration of lactose or sucrose in order to facilitate the detection of organisms that only ferment glucose. The fermentation of glucose produces a small amount of acid in the inclination of the tube, which is rapidly oxidized and the medium remains red or revert to an alkaline pH. On the other hand, the same acid reaction in the butt of the tube keeps the acid pH (yellow) due to the lower oxygen tension. When all glucose is used, organisms able to ferment lactose or sucrose will begin to utilize them. In order to enhance the free exchange of air in the slant of the tube, the tube cap must be closed loosely.

The addition of 1% Sucrose in the TSI Agar allows differentiating between Proteus and Salmonella. The fermentation of the sucrose by Proteus turns the colour of the Phenol red indicator in the slant from red to yellow. Dextrose positive and lactose negative members of the genus Salmonella, all cause a reddening of the slant and acidify the depths of the agar tubes.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 19250 and ISO 6579.

- **Composition**

Ingredients	g/L
Glucose	1.00
Lactose	10.00
Peptone	20.00
Sodium chloride	5.00
Sucrose	10.00

Ferric citrate	0.30
Bacteriological agar	12.00
Beef extract	3.00
Phenol red	0.024
Sodium thiosulfate	0.30
Yeast extract	3.00

- **Preparation**

Suspend 64.60 grams of the medium in one litre of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into tubes and sterilize in autoclave at 121 °C for 15 minutes. Allow to cool in a slanted position in order to obtain butts of 1.5-2.0 cm. depth.

- **Applications and use**

- Streak the agar slant surface and stab the butt.

- Incubate at 37 °C for 24±3 h according to ISO 6579 and 36±2 °C for 24±3 h according to ISO 19250.

- Interpret the changes in the medium as follows:

Butt:

- Yellow: Glucose positive.
- Red or unchanged: Glucose negative.
- Black: Formation of hydrogen sulphide.

Bubbles or cracks: Gas formation from glucose.

Slant surface:

- Yellow: Lactose and/or sucrose positive.
- Red or unchanged: Lactose and/or sucrose negative.

- **Quality control**

<b>Solubility</b>	w/o rests
<b>Appearance</b>	Fine powder
<b>Colour of the dehydrated medium</b>	Pink
<b>Colour of the prepared medium</b>	Red
<b>Final pH (25 °C)</b>	7.4 ± 0.2

- **Microbiological test**

According to ISO 6579: Incubation conditions: 37 °C / 24±3 h.

According to ISO 19250: Incubation conditions: 36±2 °C / 24±3 h.

Microorganism	ATCC	Specification	Characteristic reaction
<i>Shigella flexneri</i>	12022	Good growth	Slant (Red), Butt (Yellow), H <sub>2</sub> S (-), Gas (-)
<i>Salmonella enteritidis</i>	13076	Good growth	Slant (Yellow), Butt (Yellow), H <sub>2</sub> S (+), Gas (+)
<i>Proteus vulgaris</i>	13315	Good growth	Slant (Yellow), Butt (Yellow), H <sub>2</sub> S (+), Gas (+)
<i>Escherichia coli</i>	25922	Good growth	Slant (Yellow), Butt (Yellow), H <sub>2</sub> S (-), Gas (+)
<i>Pseudomonas aeruginosa</i>	9027	Good growth	Slant (Red), Butt (Red), H <sub>2</sub> S (-), Gas (-)

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

ISO 6579 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Salmonella* spp

Standard Methods for the Examination of Dairy Products. APHA, 1972.

Food and Drug Administration. Bacteriological Analytical Manual, 1976.

Vanderzant, C. and D.F. Splitt stresser (ed) 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington D.C.

European Pharmacopoeia. 4th Edition. 2002.

ISO 19250 water quality-dectetion of *Salmonella* spp.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.