## Triple Sugar Iron Agar (TSI Agar)

Art. No. 01-192

## **Specification**

Differential medium for the identification of enterobacteria, according to ISO standards 6579, 6785 and 10272.

## Formula\* in g/L

Peptone	20,000
Meat extract	3,000
Yeast extract	3,000
Lactose	10,000
Sucrose	10,000
Dextrose.	1,000
Sodium chloride	5,000
Ferric ammonium citrate	0,300
Sodium thiosulfate	0,300
Phenol red	0,025
Agar	12,000
Final nH 7.4 + 0.2 at 250C	

Final pH 7,4 ± 0,2 at 25°C

### Directions

Dissolve 64,6 g of powder in 1 L of distilled water and bring to the boil. Dispense into tubes and sterilize at 121° C for 15 minutes. Leave to solidify with short slants and good butts.

## Description

TSI Agar is a modification of the classical Kliger's agar. 1% sucrose has been added to this medium to differentiate *Proteus* and *Hafnia* (sucrose positive) from *Salmonella* and *Shigella* (sucrose negative).

Sugar degradation with acid formation is detected by turning an indicator (phenol red) to yellow, whereas alkalinization turns it to purple. When only glucose is degraded, the acid production is weak and is evaporated on the surface, so the indicator may be re-oxidised producing an alkaline surface (red) and an acid butt (yellow). If lactose or sucrose is degraded, acid production is intense and the entire medium (surface and butt) turns yellow. Gas production is detected by the formation of bubbles and occasionally cracks in the agar.

Hydrogen sulfide production, from thiosulfate or sulphured amino-acids from peptones, is detected by the formation of black FeS precipitate when the medium reacts with iron salts.

Use the medium in slanted tubes with a good butt and a short slant. Inoculate by streaking on the surface and stabbing deeply. It is advisable to use tubes with cotton plugs, in order to allow a re-oxidation of the indicator. If screw caps are used, they must be loose. See the following page for the table of reading (observations) and interpretation of results in TSI Agar.

## References

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- ISO 3560 Standard (1975) Reference Method for the Detection of Salmonella in meat and meat products.
- ISO 6579 Standard (2002) Microbiology of foods and animal feeding stuffs - Horizontal method for the detection of Salmonella spp.
- ISO 6785 Standard (2001) Milk and milk Products Detection of Salmonella spp.
- ISO 10272 Standard (1995) Microbiology of foods and animal feeding stuffs - Horizontal method for the detection of thermotolerant Campylobacter.
- ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs.- Horizontal method for the detection of Shigella spp.
- KRUMWIEDE, C. & L. KOHN (1917) A triple sugar modification of the Russell Double Sugar Medium. J. Med. Res. 37:225-229.
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## **Storage**

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place ( $+4^{\circ}$ C to 30°C and <60% RH).

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<sup>\*</sup> Adjusted and /or supplemented as required to meet performance criteria

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Genus and species	Butt	Surface	H <sub>2</sub> S Production
Escherichia coli	AG	А	-
Enterobacter aerogenes	AG	А	-
Enterobacter cloacae	AG	А	-
Citrobacter freundii	AG	А	+
Klebsiella pneumoniae	A/AG	R/K	-
Alcaligenes faecalis	R/K	R/K	-
Proteus vulgaris	AG <sup>(1)</sup>	А	+
Proteus mirabilis	AG <sup>(1)</sup>	K/A	-
Morganella morganii	AG <sup>(1)</sup>	R/K	-
Providencia	A/K	R/K	-
Salmonella typhi	Α	R/K	+ (2)
Salmonella typhimurium	AG	R/K	+
Salmonella enteritidis	A/G	R/K	+
Salmonella choleraesuis	A/G	R/K	-
Shigella spp.	Α	R/K	-
Pseudomonas aeruginosa	R/K	R/K	-

## **INTERPRETATION**

Key	•	Colour and appearance	Butt	Surface	
А		Yellow	Glucose fermentation and acid production	Lactose and/or sucrose fermentation and acid production	
G		Yellow with bubbles or cracks	Gas production from glucose		
К		Deep red	No sugar fermentation. Formation of alkaline products	No sugar fermentation. Formation of alkaline products	
R		Orange red original (No change)	No fermentation of glucose	No fermentation of lactose or sucrose	
		Blackened	H <sub>2</sub> S production		
H₂S	Not blackened	No H <sub>2</sub> S production			
	NOTES	(1) Some strains can be A without gas formation			
		(2) Only at the top of the column and sometimes only a ring after 48 hours			

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# Triple Sugar Iron Agar (TSI Agar)

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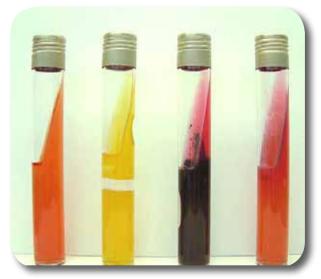
## **Quality control**

Incubation temperature:  $35^{\circ}C \pm 2,0$ 

Incubation time: 18 - 24 h

Inoculum: Stab the butt and streak the slant

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC 29212	Good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)
Shigella flexneri ATCC 12022	Good to very good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)
Proteus mirabilis ATCC 43071	Good to very good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)
Escherichia coli ATCC 25922	Good to very good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)
Salmonella typhimurium ATCC 14028	Good to very good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)
Salmonella abony NCTC 6017	Good to very good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)
Shigella sonnei ATCC 9290	Good to very good	Slant:A; Butt:K; G(-); H <sub>2</sub> S (-)



First: Uninoculated tube; Second: *E. coli* ATCC 25922; Third: *Salmonella typhimurium* ATCC 14028; Fourth: *Shigella sonnei* ATCC 9290