

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 pH 7,4 ± 0,2 at 25°C	

* Adjusted and /or supplemented as required to meet performance criteria

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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- FIL-IDF (1991) International Standard 93A. Milk and Milk Products. Detection of *Salmonella* species.
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- 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.
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- US PHARMACOPeia (2002) <61> Microbial Limit Tests. 25th ed. US Pharmacopeial Convention Inc. Rockville. Md. USA.

Storage

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

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

INTERPRETATION

Key	Colour and appearance	Butt	Surface
A	Yellow	Glucose fermentation and acid production	Lactose and/or sucrose fermentation and acid production
G	Yellow with bubbles or cracks	Gas production from glucose	
K	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
H₂S	Blackened	H ₂ S production	
	Not blackened	No H ₂ 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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Quality control

Incubation temperature: 35°C ± 2,0

Incubation time: 18 - 24 h

Inoculum: Stab the butt and streak the slant

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



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