TRIPLE SUGAR IRON AGAR (TSI)

- For in vitro use only - Catalogue No. TT95

Our TSI Agar Slants is used for the differentiation of enteric organisms (*Enterobacteriaceae*) based on carbohydrate fermentation and hydrogen sulfide production.

TSI Agar was developed by Sulkin and Willet in 1940 and is a modification of Kliger's Iron Agar. TSI Agar contains three fermentable carbohydrate sources: 0.1% dextrose, 1% sucrose, and 1% lactose. The presence of the various carbohydrates allows for differentiation between members of the *Enterobacteriaceae* based on their ability to breakdown the different carbohydrates. Peptones and extracts provide nitrogen, vitamins, amino acids, and minerals essential for bacterial growth. Phenol red acts as an indicator in the medium to detect carbohydrate fermentation. Sodium thiosulfate and ferrous sulfate are responsible for hydrogen sulfide production detection. Sodium thiosulfate acts as the substrate for enzymatic reduction and the resultant colorless hydrogen sulfide gas reacts with ferrous sulfate to produce ferrous sulfide, an insoluble black precipitate.

Formula per Litre of Medium

Beef Extract	3.0 g
Yeast Extract	3.0 g
Peptone	15.0 g
Pancreatic Digest of Animal Tissue	5.0 g
Dextrose	1.0 g
Lactose	10.0 g
Sucrose	10.0 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5.0 g
Sodium Thiosulfate	0.3 g
Agar	12.0 g
Phenol Red	0.024 g

pH 7.4 ± 0.2

Recommended Procedure

1. Allow medium to adjust to room temperature prior to inoculation.
2. Take a well-isolated colony from a pure culture plate and pick the centre using an inoculating needle.
3. Inoculate the TSI Agar Slant by stabbing the middle of the tube ¾ of the way through the butt and streaking the slant with a fishtail motion.
4. Incubate the tubes at 35°C.
5. Examine tubes and interpret results after 18 to 24 hours of incubation.

Interpretation of Results

Differentiation for TSI Agar Slants is based on carbohydrate fermentation patterns. All members of the family *Enterobacteriaceae* ferment dextrose. Organisms capable of fermenting only dextrose will result in an alkaline (red) slant and an acid (yellow) butt. Organisms capable of fermenting dextrose and lactose and/or sucrose will result in an acid (yellow) slant and acid (yellow) butt. In some instances the H_2S produced, and resultant black precipitate, may mask the acidity reaction; to overcome this phenomenon the color reaction can be examined earlier on in the incubation period (after 18 hours).

Strict aerobes, such as *Pseudomonas aeruginosa* will show only growth on the slant of the tube and not the butt, and therefore no change in the color of the butt of the tube will be observed.

Gas production is a by-product of some metabolic cycles during carbohydrate and peptone degradation, and can be observed as gas bubbles or cracks in the medium. Some organisms such as *E.*

coli may liberate an excessive amount of gas (CO_2 & H_2) resulting in the media being completely displaced to the top of the tube; therefore care should be taken when handling these tubes.

Another system of differentiation is based on H_2S production. A positive H_2S reaction appears as a black precipitate (ferrous sulfide) in the medium or a black ring near the top of the tube. It has been shown that sucrose may suppress the enzyme responsible for H_2S production therefore organisms having weak H_2S production ability may appear negative.

- *Do not stab TSI Agar Slants using an inoculating loop since the loop can crack the media giving the false appearance of gas production*

- *TSI Agar Slants must be read after 18 to 24 hours of incubation because fermentation patterns may differ for tubes incubated for shorter time periods and for prolonged periods*

Quality Control

After checking the medium for correct pH, colour, depth, and sterility, the following organisms are used to determine the performance of the completed medium.

Organism	Expected Results		
	Slant/Butt	Gas	H_2S
Escherichia coli ATCC 25922	A/A	+	–
Pseudomonas aeruginosa ATCC 27853	K/NC	–	–
Proteus mirabilis ATCC 12453	K/A	+/–	+
Salmonella typhimurium ATCC 14028	K/A	+/–	+

A = Acid (Yellow) NC = No Change (Orange)
K = Alkaline (Red)

Storage and Shelf Life

Our TSI Agar Slants should be stored in an upright position at 4°C to 8°C. Under these conditions the medium has a shelf life of 16 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
TT95-05UA	TSI Agar Slant 5-mL [16x100-mm Kim Kap]	10/pkg
TT95-07	TSI Agar Slant 7-mL [16x100-mm Kim Kap]	10/pkg
TT95-07L	TSI Agar Slant 7-mL [16x125-mm Kim Kap]	10/pkg

References

1. Sulkin SE, Willett JC. A triple sugar-ferrous sulfate medium for use in identification of enteric organisms. J Lab Clin Med 1940; 25: 649-53.
2. Hajna AA. Triple-sugar iron agar medium for the identification of intestinal group of bacteria. J Bacteriol 1945; 49:516-7.
3. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
4. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 10th ed. St. Louis: Mosby, 1998.
5. MacFaddin, J. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams and Wilkins. Philadelphia, 2000.

Original: June 2000
Revised / Revisited: October 2014