Triple Sugar Iron Agar, TSI | AS-1365

Used for differentiation of gram-negative enteric *bacilli* based on fermentation of dextrose, lactose and sucrose and hydrogen sulfide production.

Based on their ability to produce hydrogen sulfide and ferment carbohydrates, Gram-negative *Enterobacteriaceae* are biochemically characterized using Triple Sugar Iron (TSI) agar, a differential culture medium. The medium is composed of three types of carbohydrates: dextrose, sucrose, and lactose; yeast extract, which serves as a source of vitamins; and a mixture of peptone, which supplies necessary nutrients. Phenol red is used as a pH indicator; colorimetric changes indicate the presence of acidic (yellow) or alkaline (red) circumstances. To identify the creation of hydrogen sulfide, which is seen as black iron sulfide precipitates, iron salts and sodium thiosulfate are added. Osmotic balance is sustained by sodium chloride.

Similar to Kligler Iron Agar, TSI agar contains sucrose, which allows *Proteus* and *Salmonella* species to be distinguished from one another. *Proteus* fermentation of sucrose causes general acidification, whereas *Salmonella* usually ferments dextrose and then, because of peptone catabolism, returns the slant to an alkaline state.

Composition (gr/L)	
Enzymatic Digest of Casein	15
Enzymatic Digest of Animal Tissues	8
Yeast Enriched Peptone	3
Dextrose	1
Lactose	10
Sucrose	10
Ferric Ammonium Citrate	0.5
Sodium Chloride	5
Sodium Thiosulfate	0.5
Phenol Red	0.024
Agar	12
Final pH at 25°C	7.4 ± 0.2

Preparation

Dissolve 65 g of the powder into 1 liter distilled water. Autoclave at 121 °C for 15 minutes. Pour into tubes and allow to cool in slanted position.

Quality Control

Dehydrated Appearance: Pink, free-flowing, homogeneous.
Prepared Appearance: Red, slightly opalescent.
Reaction of 6.5% Solution at 25°C: pH 7.4 ± 0.2

Microbial Test Results

Incubate at 35 ± 2 °C for 18 to 24 hours with loosened caps. Use fresh cultures for inoculation and use stab and streak method.

Organism (ATCC)	**Recovery**	**Slant**	**Butt**	**Gas**	**H2S**
Escherichia coli (25922)	Good	A**	A	+	-
Pseudomonas aeruginosa (9027)	Good	K***	K	-	-

Salmonella enterica (14028)	Good	K	A	+	+
Shigella flexneri (12022)	Good	K	A	-	-
Proteus vulgaris (13315)	Good	A	A	+	+

Storage

Keep the container at 15-30 °C and prepared medium at 2-8 °C.