

Triple Sugar Iron Agar (i23183)

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

Industry: Veterinary / Food / Pharmaceutical/ Clinical

Principles & Uses

Triple Sugar Iron Agar (TSI) is a differential medium used to distinguish Gram-negative *Enterobacteria* based on their carbohydrate fermentation and H₂S production abilities. It is especially helpful in identifying these bacteria during routine bacteriological analyses, such as feces samples. TSI contains a peptone mixture for essential nutrients, including nitrogen, vitamins, minerals, and amino acids. Yeast extract contributes B-group vitamins. The medium offers three fermentable carbohydrates: Dextrose, Sucrose, and Lactose, with pH changes, indicated by Phenol red. A color shift to yellow signifies acid production, while red indicates alkalization.

TSI also contains sodium thiosulfate, which, when reduced to hydrogen sulfide, reacts with iron salt to produce black iron sulfide. Sodium chloride maintains osmotic balance, and bacteriological agar solidifies the medium. TSI's action is akin to Kligler Iron Agar but includes 1% Sucrose, enabling differentiation between *Proteus* and *Salmonella*. *Proteus* ferments sucrose, turning the Phenol red indicator in the slant from red to yellow. For *Salmonella*, which ferments Dextrose but not Lactose, the slant reddens and the agar deepens, with gas production and sometimes hydrogen sulfide. Further confirmation requires biochemical and serological tests.

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 from dehydrated Powder

Suspend 65 g of the powder in 1 Liter of distilled water. Mix thoroughly. Sterilize by autoclaving at 121°C for 15 minutes. 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

Cultural Response

Inoculate with fresh cultures by the stab and streak method and incubate with caps loosened at 35 ± 2°C for 18-24 hours.

Organism (ATCC*)	Recovery	Slant	Butt	Gas	H ₂ S
<i>Escherichia coli</i> (25922)	Good	A**	A	+	-
<i>Pseudomonas aeruginosa</i> (9027)	Good	K***	K	-	-
<i>Salmonella enterica</i> (14028)	Good	K	A	+	+
<i>Shigella flexneri</i> (12022)	Good	K	A	-	-
<i>Proteus vulgaris</i> (13315)	Good	A	A	+	+

*ATCC is a registered trade mark of the American Type Culture Collection.

**A: Color changes to yellow due to acid production.

***K: Color changes to red due to alkalization



1: Red colored prepared culture media.

2: *E. coli*, with both the butt and slant turning yellow, demonstrates acid production. When the media is displaced from the bottom, it indicates gas production.

3: *Salmonella enterica* exhibits acidity in the butt and alkalinity in the slant. This bacterium produces gas and also darkens the medium due to H₂S production.

4: *Pseudomonas aeruginosa* alkalinizes both the butt and the slant of the medium. However, it does not produce gas or H₂S.

Storage

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