B786	**TRIPLE SUGAR IRON AGAR (AS PER B.P)**

Formula

Ingredients :	**gms/lit.**
Peptone	20.00
Yeast extract	3.00
Meat extract B#	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferric ammonium citrate	0.30
Sodium thiosulphate	0.30
Phenol red	0.025
Agar	12.00

Equivalent to Beef Extract

Final pH (at 25°C) : 7.4 ± 0.2

Directions :

Suspend 64.02 gms.(Equvivalent weight of dehydrated medium per litre)in 1000 ml. distilled water. Heat to boiling for 1 min. with shaking to dissolve the medium completely. Mix well and distribute into test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the medium to set in sloped form with a butt about 1 inch long.
Note: For better results,the medium can be sterilized by autoclaving at 10 lbs pressure (115°C) for 15 minutes.

Principle :

Meat extract B, Yeast extract, Peptone provide nitrogen, vitamins, and minerals. Triple sugar iron agar contains three carbohydrates (dextrose, lactose and sucrose). When these carbohydrates are fermented, the resulting production of acid is detected by the phenol red indicator. The colour changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide. Hydrogen sulfide then reacts with an iron salt yielding the typical black iron sulfide. Sodium chloride maintains the osmotic balance of the medium. Agar is a solidifying agent.

QC Tests – (I)Dehydrated Medium	
Colour :	Light yellow to pink
Appearance :	Homogeneous Free Flowing powder
(II)Rehydrated medium	
PH (post autoclaving/heating) :	7.4 ± 0.2
Colour (post autoclaving/heating) :	Pinkish red
Clarity (post autoclaving/heating) :	Clear to slightly opalescent
(III)Q.C. Test Microbiological	

Cultural characteristics observed after 18 – 48 hrs.at 35- 37°C.

MICROORGANISM (ATCC)	GROWTH	SLANT	BUTT	GAS	H_2S
Citrobacter freundii (8090)	Luxuriant	A	A	+	+
Enterobacter aerogenes (13048)	Luxuriant	A	A	+	-
Escherichia coli (25922)	Luxuriant	A	A	+	-
Klebsiella pneumoniae (13883)	Luxuriant	A	A	+	-
Proteus vulgaris (13315)	Luxuriant	K	A	-	+
Salmonella paratyphi A	Luxuriant	K	A	+	-
Salmonella typhi (6539)	Luxuriant	K	A	-	+
Salmonella typhimurium (14028)	Luxuriant	K	A	+	+
Shigella flexneri (12022)	Luxuriant	K	A	-	-
Escherichia coli (8739)	Luxuriant	A	A	+	-
Escherichia coli (NCTC 9002)	Luxuriant	A	A	+	-

Key : A = acidic, yellow K = alkaline, no change
+ = blackening (H_2S), positive reaction
- = no reaction.

Refer disclaimer Overleaf

Precautions :	1. For Laboratory Use.				
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.				
Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
	2. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader found that not all H_2S – positive Salmonella are positive on TSI.				
	3. Sucrose is added to TSI to eliminate some sucrose – fermenting non – lactose fermenters such as Proteus and Citrobacter spp.				
	4. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.				
	5. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.				
	6. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.				
	7. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.				
Use :	For identification of gram-negative enteric bacilli on the basis of dextrose,lactose, sucrose fermentation and hydrogen sulphide production, as per B.P.				
Storage :	Dehydrated medium- below 30°C Prepared medium– Between 2 to 8°C.				
Packing :	500 gm. bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
B786	65g/l	7.692L	7.4 ± 0.2	NIL	121^0C /15 min.