BH048	**TRIPLE SUGAR IRON AGAR**
Formula	
Ingredients:	**Gms /lit.**
Peptic digest of animal tissue	10.00
Casein enzymic hydrolysate	10.00
Yeast extract	3.00
Meat Extract B#	3.00
Lactose	10.00
Sucrose	10.00
Dextrose	1.00
Sodium chloride	5.00
Ferrous sulphate	0.20
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	12.00
#- Equivalent to Beef extract	
Final pH (at 25°C) : 7.4 ± 0.2	

Directions:

Suspend 64.42 grams (the equivalent weight of dehydrated medium per Litre) in 1000 ml purified/ distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into test tubes and Sterilize by maintaining at 10lbs pressure (115°C) for 30 minutes or as per validated cycle (15 lbs pressure (121°C) for 15 minutes). Allow the medium to set in sloped form with a butt about 2.5cm long

Note: Directions specified are as per the concurrent edition of pharmacopoeia in force. Specified expiry period corresponds to this.

Principle:

Peptone, yeast extract and meat extract provide nitrogenous compounds, sulphur, trace elements and vitamin B complex etc. Sodium chloride maintains osmotic equilibrium. Triple sugar iron agar contains three fermentable 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 alkalization. . Sodium thiosulphate and ferric or ferrous ions make H2S indicator system. Sodium thiosulphate is also an inactivate or of halogen and can minimize its toxicity in the testing sample, if any during microbial limit tests. Phenol red is the pH indicator. 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 to red
	Clarity (post autoclaving/heating):	Clear to slightly opalescent
(III)Q.C. Test Microbiological		
	Cultural characteristics observed after18 – 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	+	-
	Escherichia coli (8739)	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	-	-

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

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 :	Triple Sugar Iron Agar is recommended for identification of gram-negative enteric bacilli on the basis of dextrose, lactose and sucrose fermentation and hydrogen sulphide production in accordance with Indian Pharmacopoeia. .
Storage :	Dehydrated medium- below 30 ° C Prepared mediums– Between 2 to 8°C.
Packing :	500 gm. bottle

Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
BH048	64.42g/l	7.761 L	7.4 ± 0.2	Nil	121^0C /15 min. or 115^0C /30 min.