

## Mannitol Salt Agar (Harmonized)

### Intended Use

Mannitol Salt Agar is used for the isolation and cultivation of Staphylococci in accordance with microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

### Summary

Koch reported that only Staphylococci grow on agar media containing 7.5% sodium chloride. Chapman studied this phenomenon in detail and concluded that the addition of 7.5% salt to phenol red mannitol agar results in an improved medium for the isolation of plasma coagulating Staphylococci.

The formulation of Mannitol Salt Agar is in accordance with microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP. It is used for the detection and enumeration of coagulase positive Staphylococci in milk, food and other specimens. This medium is also included in the Bacteriological Analytical Manual for cosmetics testing.

### Principle

Pancreatic digest of casein, peptic digest of animal tissue and beef extract supplies essential growth factors such as nitrogen, carbon, sulphur and trace nutrients. The 7.5% salt concentration results in partial or complete inhibition of bacteria other than Staphylococci. Mannitol fermentation, results in change in the phenol red indicator, (from red to yellow) which helps in the differentiation of staphylococcal species. Coagulase-negative species of Staphylococci and Micrococci do not ferment mannitol and grow as small red colonies surrounded by red or purple zones. Yellow coloured colonies should be tested for production of coagulase. Addition of 5% v/v Egg Yolk Emulsion enables the detection of lipase activity of staphylococci along with mannitol fermentation. The salt clears the egg yolk emulsion and lipase production is detected as yellow opaque zone around the colonies. Coagulase positive Staphylococci produce colonies surrounded by bright yellow zones while non-pathogenic staphylococci produce colonies with reddish purple zones.

### Formula\*

Ingredients	g/L
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Beef Extract	1.0
D-Mannitol	10.0
Sodium Chloride	75.0
Phenol Red	0.025
Agar	15.0
Final pH (at 25°C)	7.4 ± 0.2

\*Adjusted to suit performance parameters.

### Storage and Stability

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

### Type of Specimen

Pharmaceutical samples; Clinical samples

### Specimen Collection and Handling

Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

## Directions

1. Suspend 111.02 g of the powder in 1000 mL purified water.
2. Mix thoroughly.
3. Boil with frequent agitation to dissolve the powder completely.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. If desired, add 5% v/v Egg Yolk Emulsion (204050370100). Mix well and pour into petridishes.

**Note:** As this product contains 7.5 % sodium chloride, on repeated exposure to air and moisture absorption, it has a tendency to form lumps. Hence it is recommended to store this product in a tightly closed container in dry place away from light.

## Quality Control

**Dehydrated Appearance:** Light pink coloured, homogenous, free flowing powder.

**Prepared Appearance:** Red to rose pink coloured, slightly opalescent gel forms in petridishes.

**Growth Promotion Test:** Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18 to 72 hours.

**Growth Promoting Properties:** The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating  $\leq 100$  cfu of appropriate microorganism at 30°C-35°C for 18 hours.

**Indicative Properties:** The test results observed are within the specified temperature and time, inoculating  $\leq 100$  cfu of appropriate microorganism.

**Inhibitory Properties:** No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating  $>100$  cfu of the appropriate microorganism at 30°C-35°C for 72 hours.

Organism (ATCC)	Growth	Colour of Colony
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> (6538)	Good	Yellow colonies surrounded by yellow zone

Inhibitory		
<i>Escherichia coli</i> (8739)	Inhibited	–

**Note:** For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum. For inhibition no growth of test microorganism should occur.

## Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

## Precautions / Limitations

The medium is recommended for the isolation of marine bacteria. Further biochemical and serological testing must be carried out for further identification.

## Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

## Reference

1. Chapman, 1945. J. Bact; 50:201.
2. The United States Pharmacopoeia, 2023, The United States Pharmacopoeial Convention. Rockville, MD.
3. British Pharmacopoeia, 2023, The Stationery Office British Pharmacopoeia.
4. European Pharmacopoeia, 2011, European Dept. for the quality of Medicines.
5. Japanese Pharmacopoeia, 2008.
6. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8<sup>th</sup> Ed; Rev. A, AOAC, International, Gaithersburg, Md.
7. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

**Product Presentation:**

<b>Cat No.</b>	<b>Product description</b>	<b>Pack Size</b>
201130370500	Dehydrated Culture Media	500 g
201130372500	Dehydrated Culture Media	2.5 k
201130375000	Dehydrated Culture Media	5 k
205130930100	Ready Prepared Plate	(90 mm) 100 Plates

**Disclaimer**

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.

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