

## Directions for Preparation from Dehydrated Product

### Difco™ Malt Extract Agar

1. Suspend 33.6 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.
4. Test samples of the finished product for performance using stable, typical control cultures.

### Difco™ Malt Extract Broth

1. Dissolve 15 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

See appropriate references for specific procedures.

## Expected Results

Refer to appropriate references and procedures for results.

## References

1. Reddish. 1919. *Abstr. Bacteriol.* 3:6.
2. Thom and Church. 1926. *The aspergilli*. Williams & Wilkins, Baltimore, Md.
3. U.S. Food and Drug Administration. 1995. *Bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, Md.

## Availability

### Difco™ Malt Extract Agar

Cat. No. 211220 Dehydrated – 500 g

### Europe

Cat. No. 254487 Prepared Plates – Pkg. of 20\*

### Difco™ Malt Extract Broth

#### BAM

Cat. No. 211320 Dehydrated – 500 g

\*Store at 2-8°C.

# Mannitol Salt Agar

## Intended Use

This medium conforms with specifications of *The United States Pharmacopeia (USP)*.

Mannitol Salt Agar is used for the selective isolation and enumeration of staphylococci from clinical and nonclinical materials.

## Summary and Explanation

Koch, in 1942, reported that only staphylococci grow on agar media containing 7.5% sodium chloride.<sup>1</sup> Chapman further studied this phenomenon in greater detail and concluded that the addition of 7.5% sodium chloride to phenol red mannitol

agar results in an improved medium for the isolation of plasma-coagulating staphylococci.<sup>2</sup> This medium is listed as one of several recommended for the enumeration of gram-positive bacteria in cosmetics<sup>3</sup> and is recommended in the *USP* for use in the performance of Microbial Limit Tests.<sup>4</sup>

## Principles of the Procedure

Mannitol Salt Agar is a nutritive medium due to its content of peptones and beef extract, which supply essential growth factors, such as nitrogen, carbon, sulfur and trace nutrients. The 7.5% concentration of sodium chloride results in the partial or complete inhibition of bacterial organisms other than staphylococci. Mannitol fermentation, as indicated by a change in the phenol red indicator, aids in the differentiation of staphylococcal species.

## Formulae

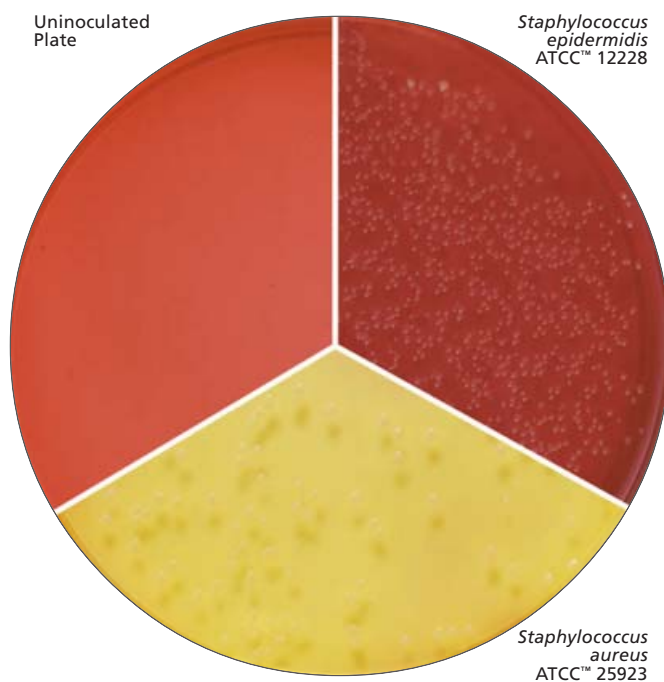
### Difco™ Mannitol Salt Agar

Approximate Formula* Per Liter	
Proteose Peptone No. 3 .....	10.0 g
Beef Extract .....	1.0 g
D-Mannitol .....	10.0 g
Sodium Chloride .....	75.0 g
Agar .....	15.0 g
Phenol Red .....	25.0 mg

### BBL™ Mannitol Salt Agar

Approximate Formula* Per Liter	
Pancreatic Digest of Casein .....	5.0 g
Peptic Digest of Animal Tissue .....	5.0 g
Beef Extract .....	1.0 g
D-Mannitol .....	10.0 g
Sodium Chloride .....	75.0 g
Agar .....	15.0 g
Phenol Red .....	25.0 mg

\*Adjusted and/or supplemented as required to meet performance criteria.



## User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

### Identity Specifications

#### Difco™ Mannitol Salt Agar

Dehydrated Appearance:	Light pink, free-flowing, homogeneous.
Solution:	11.1% solution, soluble in purified water upon boiling. Solution is red, slightly opalescent.
Prepared Appearance:	Pinkish red, slightly opalescent.
Reaction of 11.1% Solution at 25°C:	pH 7.4 ± 0.2

### Cultural Response

#### Difco™ Mannitol Salt Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours or up to 48 hours. A yellow zone around the colony indicates mannitol has been fermented.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM AROUND COLONY
<i>Enterobacter aerogenes</i>	13048	10 <sup>3</sup>	Marked to complete inhibition	–
<i>Escherichia coli</i>	25922	10 <sup>3</sup>	Marked to complete inhibition	–
<i>Proteus mirabilis</i>	12453	10 <sup>3</sup>	Partial inhibition	–
<i>Staphylococcus aureus</i>	25923	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good	Yellow
<i>Staphylococcus epidermidis</i>	12228	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good	Red

### Identity Specifications

#### BBL™ Mannitol Salt Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many light to dark red flecks.
Solution:	11.1% solution, soluble in purified water upon boiling. Solution is medium to dark, red to rose, clear to slightly hazy.
Prepared Appearance:	Medium to dark, red to rose, clear to slightly hazy.
Reaction of 11.1% Solution at 25°C:	pH 7.4 ± 0.2

### Cultural Response

#### BBL™ Mannitol Salt Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 42-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF MEDIUM AROUND COLONY
<i>Proteus mirabilis</i>	12453	10 <sup>4</sup> -10 <sup>5</sup>	Partial to complete inhibition	–
<i>Staphylococcus aureus</i>	25923	10 <sup>3</sup> -10 <sup>4</sup>	Good	Yellow
<i>Staphylococcus epidermidis</i>	12228	10 <sup>3</sup> -10 <sup>4</sup>	Good	Red

## Directions for Preparation from Dehydrated Product

1. Suspend 111 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

Use standard procedures to obtain isolated colonies from specimens. Incubate plates for 24-48 hours at 35 ± 2°C in an aerobic atmosphere.

## Expected Results

Typical colonial morphology on Mannitol Salt Agar is as follows:

<i>Staphylococcus aureus</i> .....	Small to large with yellow zones
Staphylococci other than <i>S. aureus</i> .....	Small to large with red zones
Streptococci .....	No growth to trace growth
Micrococci .....	Large, white to orange
Gram-negative bacteria .....	No growth to trace growth

## References

1. Koch. 1942. Zentralbl. Bakteriol. Parasitenkd. Abt. I Orig. 149:122.
2. Chapman. 1945. J. Bacteriol. 50:201.
3. Hitchens, Tran and McCarron. 1995. FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
4. United States Pharmacopeial Convention, Inc. 2001. The United States pharmacopeia 25/The national formulary 20 – 2002. United States Pharmacopeial Convention, Inc., Rockville, Md.

## Availability

### Difco™ Mannitol Salt Agar

<b>BAM</b>	<b>BS10</b>	<b>CMPH</b>	<b>MCM7</b>	<b>USP</b>
Cat. No.	230650	Dehydrated – 500 g		
	230620	Dehydrated – 2 kg		
	230630	Dehydrated – 10 kg		

### BBL™ Mannitol Salt Agar

<b>BAM</b>	<b>BS10</b>	<b>CMPH</b>	<b>MCM7</b>	<b>USP</b>
Cat. No.	211407	Dehydrated – 500 g		
	211410	Dehydrated – 5 lb (2.3 kg)		
	293689	Dehydrated – 25 lb (11.3 kg)		

#### United States and Canada

Cat. No.	221173	Prepared Plates – Pkg. of 20*
	221271	Prepared Plates – Ctn. of 100*

#### Europe

Cat. No.	254027	Prepared Plates – Pkg. of 20*
	254079	Prepared Plates – Ctn. of 120*

#### Japan

Cat. No.	251173	Prepared Plates – Pkg. of 20*
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\*Store at 2-8°C.