

# PRODUCT SPECIFICATION SHEET



## MacConkey Agar w/o CV, NaCl w/ 0.5% Bile Salts (DM149)

### Intended Use

MacConkey Agar w/o CV, NaCl w/ 0.5% Bile salts (DM149) is recommended for cultivation and differentiation of enteric bacteria, restricting *Proteus* swarming from urine specimens which may contain large number of *Proteus* species as well as potentially pathogenic gram-positive organisms.

### Product Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.<sup>(1)</sup> MacConkey Agar is the earliest selective and differential medium for cultivation of enteric microorganisms from a variety of clinical specimens.<sup>(1, 2)</sup> Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs<sup>(3)</sup> and for direct plating / inoculation of water samples for coliform counts.<sup>(4)</sup> These media are also accepted by the Standard Methods for the Examination of Milk and Dairy Products<sup>(5, 6)</sup> and pharmaceutical preparations. The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Modifications made in the original formula improved the growth of *Shigella* and *Salmonella* strains. MacConkey Agar is listed as one of the recommended media for the isolation of *E. coli* from nonsterile pharmaceutical products.<sup>(7)</sup>

MacConkey Agar w/o Crystal Violet & Salt is a differential medium that restricts swarming of *Proteus* spp., aiding in the detection and isolation of enteric microorganisms. Sodium Chloride is deleted from the medium to provide an electrolyte deficient medium preventing *Proteus* spp. from spreading. In addition, this medium does not contain crystal violet allowing *Staphylococcus*, *Enterococcus*, and *Mycobacterium* spp. to grow. MacConkey Agar contains bile salts which attributes to the selective action to this medium, that inhibit gram-positive organisms and allow gram-negative organisms to grow. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile. The red colour is due to production of acid from lactose, absorption of neutral red and a subsequent colour change of the dye when the pH of medium falls below 6.8. Lactose non-fermenting strains, such as *Shigella* and *Salmonella* are colourless and transparent and typically do not alter appearance of the medium. When lactose non-fermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas.

### Principles of the Procedure

Peptic digest of animal tissue in MacConkey Agar w/o Crystal Violet & Salt, serves as the main source of nitrogen and vitamins. Lactose serves as the carbon source by being the fermentable carbohydrate. Bile salts serve to make the medium selective by inhibiting accompanying gram-positive bacteria. Neutral red is the pH indicator dye.

### Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	20.00
Lactose	10.00
Bile salts	5.00
Neutral red	0.075
Agar	12.00
Final pH: 7.4 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

### Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

### Directions

1. Suspend 47 grams of the medium in one liter of distilled water.
2. Heat if necessary, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle. Cool to 45-50°C
4. Mix well and pour into sterile petri plates.

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5. DO NOT OVERHEAT.
6. The surface of the medium should be dry when inoculated.

## Quality Control Specifications

Dehydrated Appearance	Light yellow to pink homogeneous free flowing powder
Prepared Medium	Orange red coloured clear to slightly opalescent gel forms in Petri plates
Reaction of 4.7% Solution	pH : 7.4 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.2% Agar gel

**Expected Cultural Response:** Cultural characteristics observed after an incubation 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Recovery	Colour of colony
1.	<i>Escherichia coli</i> ATCC 25922	50 -100	good-luxuriant	≥50 %	pink to red with bile precipitate
2.	<i>Enterobacter aerogenes</i> ATCC 13048	50 -100	good-luxuriant	≥50 %	pink to red
3.	<i>Enterococcus faecalis</i> ATCC 29212	50-100	fair to good	30-40%	pale pink to red
4.	<i>Proteus vulgaris</i> ATCC 13315	50 -100	good-luxuriant	≥50 %	Colourless
5.	<i>Salmonella Paratyphi A</i> ATCC 9150	50 -100	good-luxuriant	≥50 %	Colourless
6.	<i>Shigella flexneri</i> ATCC 12022	50 -100	fair to good	30-40%	Colourless
7.	<i>Salmonella Paratyphi B</i> ATCC 8759	50 -100	good-luxuriant	≥50 %	Colourless
8.	<i>Salmonella Enteritidis</i> ATCC 13076	50 -100	good-luxuriant	≥50 %	Colourless
9.	<i>Salmonella Typhi</i> ATCC 6539	50 -100	good-luxuriant	≥50 %	Colourless
10.	<i>Staphylococcus aureus</i> ATCC 25923	50 -100	fair to good	30-40%	pale pink -red

The organisms listed are the minimum that should be used for quality control testing.

## Test Procedure

Refer to appropriate references using MacConkey Agar w/o CV and NaCl for the isolation and identification of enteric organisms.

## Results

1. Lactose-fermenting organisms grow as pink to red colonies with or without a zone of precipitated bile.
2. Non-lactose fermenting organisms grow as colorless or clear colonies.
3. Swarming by *Proteus* spp. is reduced.

## Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

## Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

## Limitations of the Procedure

1. Although MacConkey Agar w/o Crystal Violet & Salt is a selective medium, it is less inhibitory than MacConkey Agar, allowing gram-positive organisms to grow, biochemical and serological testing using pure cultures are recommended for complete identification.
2. Consult appropriate texts for detailed information and recommended procedures.
3. Incubation of MacConkey Agar w/o Crystal Violet & Salt plates under increased CO<sub>2</sub> has been reported to reduce the growth and recovery of certain gram-negative bacilli.

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## Packaging

**Product Name :** MacConkey Agar w/o CV, NaCl w/ 0.5% Bile salts.

**Product Code :** DM149

**Available Pack sizes :** 100gm / 500gm

## References

1. MacConkey, 1905, J. Hyg., 5:333.
2. MacConkey, 1900, The Lancet, ii:20.
3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
4. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed., APHA, Washington, D.C.
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. The United States Pharmacopoeia XXI and the National Formulary, 16th ed., 1985, United States Pharmacopoeial Convention, Inc., Washington, D.C.
7. United States Pharmacopoeial Convention, Inc. 2008. The United States pharmacopoeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopoeial Convention, Inc., Rockville, Md.

## Further Information

For further information please contact your local MICROMASTER Representative.



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