MacConkey Agars
MacConkey Agar • MacConkey Agar Base
MacConkey Agar without Crystal Violet
MacConkey Agar without Crystal Violet or Salt
MacConkey Agar without Salt

Intended Use

MacConkey agars are slightly selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical,[1-3] dairy,[4] food,[5-7] water,[8] pharmaceutical,[9-11] cosmetic,[6,7] and other industrial sources.

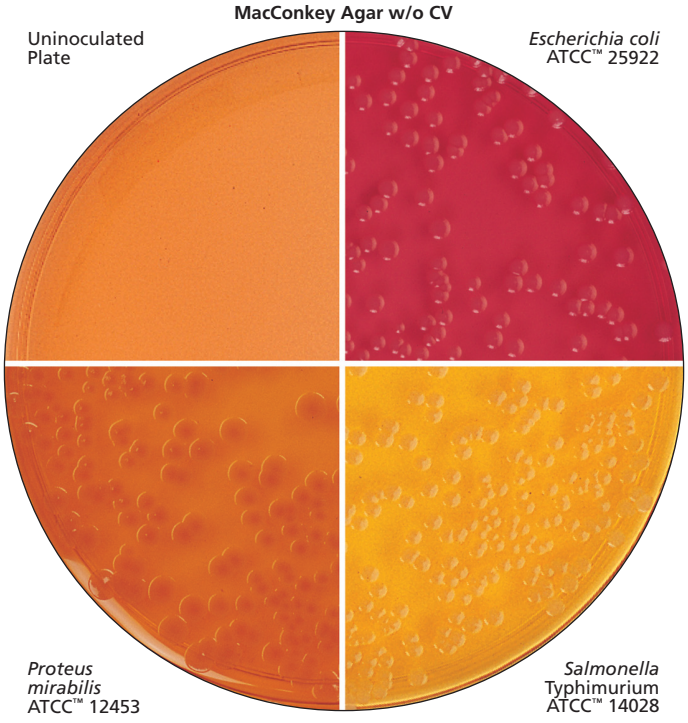
MacConkey Agar is used for isolating and differentiating lactose-fermenting from lactose-nonfermenting gram-negative enteric bacilli.

MacConkey Agar Base is used with added carbohydrate in differentiating coliforms based on fermentation reactions.

MacConkey Agar without Crystal Violet is used for isolating and differentiating enteric microorganisms while permitting growth of staphylococci and enterococci. The medium can be used also to separate *Mycobacterium fortuitum* and *M. chelonae* from other rapidly growing mycobacteria.

MacConkey Agar without Crystal Violet or Salt and MacConkey Agar without Salt are used for isolating and differentiating gram-negative bacilli while suppressing the swarming of most *Proteus* species.

MacConkey Agar meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*[9-11] performance specifications, where applicable.



Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.[12]

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group.

MacConkey Agar contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose nonfermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas. MacConkey Agar is listed as one of the recommended media for the isolation of *E. coli* from nonsterile pharmaceutical products.[9]

MacConkey Agar Base is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

MacConkey Agar without Crystal Violet is a differential medium that is less selective than MacConkey Agar. The lack of crystal violet permits the growth of *Staphylococcus* and *Enterococcus*. Staphylococci produce pale pink to red colonies and enterococci produce compact tiny red colonies either on or beneath the surface of the medium. The medium is used also to separate *Mycobacterium fortuitum* and *M. chelonae* from other rapidly growing mycobacteria.[13,14]

MacConkey Agar without Crystal Violet or Salt and MacConkey Agar without Salt (which also lacks crystal violet) are differential media used for isolating and cultivating gram-negative enteric organisms and gram-positive cocci from waters, feces and other sources suspected of containing these organisms, as well as limiting the swarming of *Proteus* species.

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™ MacConkey Agar

Dehydrated Appearance: Pink to pinkish beige, free-flowing, homogeneous.

Solution: 5.0% solution, soluble in purified water upon boiling. Solution is reddish-purple, slightly opalescent.

Prepared Appearance: Pinkish red, slightly opalescent.

Reaction of 5.0% Solution at 25°C: pH 7.1 ± 0.2

Difco™ MacConkey Agar Base

Dehydrated Appearance: Pinkish beige, free-flowing, homogeneous.

Solution: 4.0% solution, soluble in purified water upon boiling. Solution is red, very slightly to slightly opalescent.

Prepared Appearance: Red, slightly opalescent.

Reaction of 4.0% Solution at 25°C: pH 7.1 ± 0.2

Difco™ MacConkey Agar without Crystal Violet

Dehydrated Appearance: Pinkish beige, free-flowing, homogeneous.

Solution: 5.2% solution, soluble in purified water upon boiling. Solution is reddish orange, clear to very slightly opalescent.

Prepared Appearance: Reddish orange, clear to very slightly opalescent.

Reaction of 5.2% Solution at 25°C: pH 7.4 ± 0.2

Difco™ MacConkey Agar without Salt

Dehydrated Appearance: Beige to pinkish beige, free-flowing, homogeneous.

Solution: 4.7% solution, soluble in purified water upon boiling. Solution is reddish orange, slightly opalescent.

Prepared Appearance: Reddish orange, slightly opalescent.

Reaction of 4.7% Solution at 25°C: pH 7.4 ± 0.2

Cultural Response

Difco™ MacConkey Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours (incubate *E. coli* ATCC 25922 for 40-48 hours). For *E. coli* ATCC 8739, inoculate in duplicate and incubate one plate at 30-35°C for 18-24 hours and the other plate at 35-37°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–	–
Escherichia coli	25922	30-300	Good	Pink to red	+
Proteus mirabilis	12453	30-300	Good	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	–
Escherichia coli	8739	<100	Growth (18-24 hours at 30-35°C)	Pink to red	+
Escherichia coli	8739	<100	Growth (18-72 hours at 35-37°C)	Pink to red	+

Difco™ MacConkey Agar Base

Prepare the medium per label directions without and with 1% added lactose. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR PLAIN	COLONY COLOR W/LACTOSE	BILE PPT.
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–	–	–
Escherichia coli	25922	30-300	Good	Colorless	Pink to red	+ (w/lactose)
Proteus mirabilis	12453	30-300	Good	Colorless	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	Colorless	–

Difco™ MacConkey Agar without Crystal Violet

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	30-300	Good	Red	–
Escherichia coli	25922	30-300	Good	Pink to red	–
Proteus mirabilis	12453	30-300	Good	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	–
Staphylococcus aureus	25923	30-300	Good	Pink to red	–

Difco™ MacConkey Agar without Salt

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	33186	30-300	Good	Red	–
Escherichia coli	25922	30-300	Good	Pink to red	–
Proteus mirabilis	12453	30-300	Good	Colorless, no swarming	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	30-300	Good	Colorless	–
Shigella flexneri	12022	30-300	Good	Colorless	–

Continued

Identity Specifications

BBL™ MacConkey Agar

Dehydrated Appearance: Fine, homogenous, may contain dark particles.

Solution: 5.0% solution, soluble in purified water upon boing. Solution is medium to dark, rose to brown-rose with or without a trace orange tint; clear to slightly hazy.

Prepared Appearance: Medium to dark, rose to brown-rose with or without a trace orange tint; clear to slightly hazy.

Reaction of 5.0% Solution at 25°C: pH 7.1 ± 0.2

BBL™ MacConkey Agar (prepared)

Appearance: Medium-dark, rose-tan and trace hazy.

Reaction at 25°C: pH 7.1 ± 0.2

BBL™ MacConkey Agar without Crystal Violet

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 5.2% solution, soluble in purified water upon boiling. Solution is medium, red-orange to red-rose, slightly hazy to hazy.

Prepared Appearance: Medium, red-orange to red-rose, slightly hazy to hazy.

Reaction of 5.2% Solution at 25°C: pH 7.4 ± 0.2

BBL™ MacConkey Agar without Crystal Violet or Salt

Dehydrated Appearance: Fine, homogeneous, free of extraneous material.

Solution: 4.37% solution, soluble in purified water upon boiling. Solution is medium, red-orange to red-rose, slightly hazy to hazy.

Prepared Appearance: Medium, red-orange to red-rose, slightly hazy to hazy.

Reaction of 4.37% Solution at 25°C: pH 7.4 ± 0.2

Cultural Response

BBL™ MacConkey Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 48 hours. For *E. coli* ATCC 8739, inoculate in duplicate and incubate one plate at 30-35°C for 18-24 hours and the other plate at 35-37°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–	–
Escherichia coli	25922	10^3-10^4	Good	Red to rose-red	+
Proteus mirabilis	12453	10^3-10^4	Good	Colorless	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–
Shigella flexneri	12022	10^3-10^4	Good	Colorless	–
Escherichia coli	8739	<100	Growth (18-24 hours at 30-35°C)	Red to rose-red	+
Escherichia coli	8739	<100	Growth (18-72 hours at 35-37°C)	Red to rose-red	+

BBL™ MacConkey I Agar (prepared)

Inoculate and incubate at 35 ± 2°C for 18-24 hours. Incubate *E. coli* ATCC 8739 at 30-35°C for 18-72 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^4-10^5	Partial to complete inhibition	–	–
Escherichia coli	25922	10^3-10^4	Good	Red to rose-red	+
Pseudomonas aeruginosa	10145	10^3-10^4	Good	Greenish yellow	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	No reaction	–
Shigella dysenteriae	9361	10^3-10^4	Good	No reaction	–
Escherichia coli	8739	10-100	Growth	Red to rose-red	+

BBL™ MacConkey Agar without Crystal Violet

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary (up to 11 days for *M. fortuitum*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-10^4	Good	Rose red	–
Escherichia coli	25922	10^3-10^4	Good	Pink to rose red	–
Mycobacterium fortuitum	6841	10^3-10^4	Good	Rose red	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–
Staphylococcus aureus	25923	10^3-10^4	Good	Pink to rose red	–

BBL™ MacConkey Agar without Crystal Violet or Salt

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary (up to 11 days for *M. fortuitum*).

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
Enterococcus faecalis	29212	10^3-10^4	Good	Rose red	–
Escherichia coli	25922	10^3-10^4	Good	Pink to rose red	–
Proteus mirabilis	12453	10^3-10^4	Good	Colorless, no swarming	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3-10^4	Good	Colorless	–

Principles of the Procedure

Peptones are sources of nitrogen and other nutrients. Yeast extract is a source of trace elements, vitamins, amino acids and carbon. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile salts, bile salts no. 3, oxgall and crystal violet are selective agents that inhibit growth of gram-positive organisms. Sodium chloride maintains osmotic balance in the medium. Magnesium sulfate is a source of divalent cations. Agar is the solidifying agent.

Formulae

Difco™ MacConkey Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Peptones (meat and casein)	3.0	g
Lactose	10.0	g
Bile Salts No. 3	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

Difco™ MacConkey Agar Base

Consists of the same ingredients without the lactose.

BBL™ MacConkey Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Peptones (meat and casein)	3.0	g
Lactose	10.0	g
Bile Salts	1.5	g
Sodium Chloride	5.0	g
Agar	13.5	g
Neutral Red	0.03	g
Crystal Violet	1.0	mg

Difco™ MacConkey Agar without Crystal Violet

Approximate Formula* Per Liter

Peptone	20.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g
Neutral Red	0.05	g

BBL™ MacConkey Agar without Crystal Violet

Approximate Formula* Per Liter

Pancreatic Digest of Casein	10.0	g
Peptic Digest of Animal Tissue	10.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Sodium Chloride	5.0	g
Agar	12.0	g
Neutral Red	0.05	g

Difco™ MacConkey Agar without Salt

Approximate Formula* Per Liter

Peptone	20.0	g
Lactose	10.0	g
Bile Salts	5.0	g
Agar	12.0	g
Neutral Red	75.0	mg

BBL™ MacConkey Agar without Crystal Violet or Salt

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	10.0	g
Yeast Extract	10.0	g
Lactose	10.0	g
Oxgall	5.0	g
Magnesium Sulfate	0.2	g
Agar	12.0	g
Neutral Red	75.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ MacConkey Agar – 50 g;
   BBL™ MacConkey Agar – 50 g;
   Difco™ MacConkey Agar Base – 40 g;
   Difco™ MacConkey Agar without Crystal Violet – 52 g;
   BBL™ MacConkey Agar without Crystal Violet – 52 g;
   BBL™ MacConkey Agar without Crystal Violet or Salt – 47.3 g;
   Difco™ MacConkey Agar without Salt – 47 g.
   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.

NOTE: If MacConkey Agar Base is to be used within 12 hours, omit autoclaving and gently boil medium for 5 minutes. Add 1% carbohydrate before or after autoclaving, depending upon heat lability. The surface of MacConkey agars without salt should be thoroughly air-dried prior to inoculation.

4. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For clinical specimens, refer to laboratory procedures for details on specimen collection and handling.[1-3]

For food or dairy samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[4-7]

For cosmetics, water, or other industrial samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.[6-11]

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *E. coli* using MacConkey Agar.[9]

Procedure

Refer to appropriate standard references for details on test methods to obtain isolated colonies from specimens or samples using MacConkey Agar.[1-11] Incubate plates for 18-72 hours at 35 ± 2°C under appropriate atmospheric conditions, or as instructed in the standard reference.[1-11]

Expected Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or without a zone of precipitated bile. Lactose-nonfermenting organisms grow as colorless or clear colonies.

Swarming by *Proteus* spp. is reduced on MacConkey agars without salt.

On MacConkey Agar without Crystal Violet and MacConkey agars without salt, staphylococci produce pale pink to red colonies and enterococci produce tiny red colonies; these organisms are inhibited on MacConkey Agar.

On MacConkey Agar without Crystal Violet, potentially pathogenic rapid growers of the *M. fortuitum* complex usually grow in 5-11 days, while the commonly saprophytic species are inhibited.[3,13]

On MacConkey agars without salt, the swarming of *Proteus* is reduced.

Limitations of the Procedure

1. Although MacConkey media are selective primarily for gram-negative enteric bacilli, biochemical and, if indicated, serological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,3]
2. Incubation of MacConkey Agar plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.[14]
3. Some strains of *M. smegmatis* from humans may grow on MacConkey Agar without Crystal Violet, but these strains can be differentiated from *M. fortuitum* complex by the 3-day arylsulfatase test.[9]

References

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Availability

Difco™ MacConkey Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD SMWW USP

Cat. No.	212123	Dehydrated – 500 g†
	212122	Dehydrated – 2 kg†
	275300	Dehydrated – 10 kg†

BBL™ MacConkey Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD SMWW USP

Cat. No.	211387	Dehydrated – 500 g†
	211390	Dehydrated – 5 lb (2.3 kg)†
	211391	Dehydrated – 25 lb (11.3 kg)†

BBL™ MacConkey I Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP JP MCM9 SMD SMWW USP

United States and Canada

Cat. No.	215197	Prepared Plates – Pkg. of 20*†
	297064	Prepared Plates – Ctn. of 100*†

Difco™ MacConkey Agar Base

Cat. No. 281810 Dehydrated – 500 g

Difco™ MacConkey Agar without Crystal Violet

Cat. No. 247010 Dehydrated – 500 g

BBL™ MacConkey Agar without Crystal Violet

Cat. No. 211393 Dehydrated – 500 g

Europe

Cat. No. 256008 Prepared Plates – Pkg. of 20*

BBL™ MacConkey Agar without Crystal Violet or Salt

Cat. No. 294584 Dehydrated – 500 g

Difco™ MacConkey Agar without Salt

Cat. No.	233120	Dehydrated – 500 g
	233110	Dehydrated – 10 kg

Europe

Cat. No.	256009	Prepared Plates – Pkg. of 20*
	257286	Prepared Plates – Ctn. of 120*

** Store at 2-8°C.*
† QC testing performed according to USP/EP/JP performance specifications.