

MacConkey II Agar • MacConkey II Agar with MUG

Intended Use

MacConkey II Agar is a slightly selective and differential medium for the detection of coliform organisms and enteric pathogens.

MacConkey II Agar with MUG is used for the presumptive identification of *Escherichia coli*.

MacConkey II Agar meets *United States Pharmacopeia (USP)* performance specifications.

Summary and Explanation

The BBL™ MacConkey II Agar formulation was made available in 1983. It was specially designed to improve the inhibition of swarming *Proteus* species, to achieve more definitive differentiation of lactose fermenters and nonfermenters, and for the promotion of superior growth of enteric pathogens.

Trepeta and Edberg¹ modified MacConkey Agar by the incorporation of MUG (4-methylumbelliferyl-β-D-glucuronide). The resulting medium allowed the authors to presumptively identify *E. coli* from the primary plating medium within 5 minutes.

Principles of the Procedure

MacConkey II Agar is a selective and differential medium. It is only slightly selective since the concentration of bile salts, which inhibit gram-positive microorganisms, is low in comparison with other enteric plating media. Crystal violet also is included in the medium to inhibit the growth of gram-positive bacteria, especially enterococci and staphylococci.

Differentiation of enteric microorganisms is achieved by the combination of lactose and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate.

Most strains (96-97%) of *E. coli* produce β-D-glucuronidase.² The enzyme hydrolyzes MUG to yield 4-methylumbelliferone, a compound that fluoresces under long-wave (366 nm) UV light. The addition of MUG to the formulation allows β-D-glucuronidase-positive strains of *E. coli* to fluoresce blue-green when examined under UV light.

BBL MacConkey II Agar with MUG contains 0.1 g of MUG per liter of MacConkey II Agar.

Formula

BBL™ MacConkey II Agar

Approximate Formula* Per Liter

Pancreatic Digest of Gelatin	17.0	g
Pancreatic Digest of Casein	1.5	g
Peptic Digest of Animal Tissue.....	1.5	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

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

User Quality Control

Identity Specifications

BBL™ MacConkey II Agar

Dehydrated Appearance:	Fine, homogeneous, may contain dark particles.
Solution:	5.0% solution, soluble in purified water upon boiling. Solution is medium to dark, rose to brown-rose, clear to slightly hazy.
Prepared Appearance:	Medium to dark, rose to brown-rose, clear to slightly hazy.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

BBL™ MacConkey II Agar

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	BILE PPT.
<i>Enterococcus faecalis</i>	29212	10 ⁴ -10 ⁵	Partial to complete inhibition	–	–
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	Pink to red	+
<i>Proteus mirabilis</i>	12453	10 ³ -10 ⁴	Good, inhibition of swarming	Colorless	–
<i>Pseudomonas aeruginosa</i>	10145	10 ³ -10 ⁴	Good	Colorless to blue to green to pink	–
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Colorless	–



Directions for Preparation from Dehydrated Product

1. Suspend 50 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. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Incubate plates, protected from light, at 35 ± 2°C (do not use CO₂-enriched atmosphere with MacConkey II Agar) or other appropriate temperature for 18-24 hours; if negative after 24 hours on MUG-containing medium, reincubate an additional 24 hours.

Expected Results

After incubation, examine the medium macroscopically for typical colonies. Colonies of lactose-fermenting bacteria appear pink to rose-red in color and may be surrounded by a zone of bile precipitation, while lactose-nonfermenting colonies are colorless.

Examine MacConkey II Agar with MUG under long-wavelength UV light (366 nm). β-D-glucuronidase-positive colonies have a blue-green fluorescence; β-D-glucuronidase-negative colonies do not fluoresce.

Limitations of the Procedure

Not all strains of *E. coli* ferment lactose or produce β-D-glucuronidase. Some strains of *Salmonella* and *Shigella* produce β-D-glucuronidase and will fluoresce.³ A small percentage of *Yersinia* and streptococci have been reported to fluoresce.⁴ Additional biochemical or serological tests are necessary for definitive identification.

References

1. Trepeta and Edberg. 1984. *J. Clin. Microbiol.* 19:172.
2. Killian and Bulow. 1976. *Acta Pathol. Microbiol. Scand. Sec. B.* 84:245.
3. Feng and Hartman. 1982. *Appl. Environ. Microbiol.* 43:1320.
4. Robison. 1984. *Appl. Environ. Microbiol.* 48:285.

Availability

BBL™ MacConkey II Agar

AOAC BAM BS12 CCAM CMPH2 COMPF EP MCM9 SMD
SMWW USP

Cat. No. 212306 Dehydrated – 500 g
292861 Dehydrated – 10 kg

United States and Canada

Cat. No. 221172 Prepared Plates – Pkg. of 20*
221270 Prepared Plates – Ctn. of 100*

Europe

Cat. No. 254025 Prepared Plates – Pkg. of 20*
254078 Prepared Plates – Ctn. of 120*

Japan

Cat. No. 251172 Prepared Plates – Pkg. of 20*
251270 Prepared Plates – Ctn. of 100*
251577 Prepared Plates (Deep Fill) – Ctn. of 100*

BBL™ MacConkey II Agar//Columbia CNA Agar with 5% Sheep Blood

BS12 CMPH2 MCM9

Cat. No. 221600 Prepared I Plate™ Dishes – Pkg. of 20*
221601 Prepared I Plate™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

BS12 CMPH2 MCM9

United States and Canada

Cat. No. 221290 Prepared I Plate™ Dishes – Pkg. of 20*
221291 Prepared I Plate™ Dishes – Ctn. of 100*

Europe

Cat. No. 251290 Prepared I Plate™ Dishes – Pkg. of 20*

BBL™ MacConkey II Agar//Levine EMB Agar

Cat. No. 295969 Prepared I Plate™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar//Columbia PNA Agar with 5% Sheep Blood

Cat. No. 297272 Prepared I Plate™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar//Chocolate II Agar//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No. 299580 Prepared Y Plate™ Dishes – Ctn. of 100*

BBL™ MacConkey II Agar with MUG

Cat. No. 221938 Prepared Plates – Pkg. of 20*

BBL™ MacConkey II Agar with MUG//Trypticase™ Soy Agar with 5% Sheep Blood (TSA II)

Cat. No. 221949 Prepared I Plate™ Dishes – Pkg. of 20*

*Store at 2-8°C.