

Instructions for Use

MACCONKEY AGAR

Cat. no. GA35	MacConkey Agar, 15x100mm Plate, 19ml (reduced stacking ring)	10 plates/bag
Cat. no. Q63	MacConkey Agar, 20x150mm, 20ml Deep	20 tubes/box
Cat. no. J32	Blood Agar / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J32BX	Blood Agar / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	100 plates/box
Cat. no. J58	MacConkey Agar / EMB Agar, Levine, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J62	Columbia CNA Agar / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J62BX	Columbia CNA Agar / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	100 plates/box
Cat. no. J85	Rose Agar / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J118	MacConkey Agar / MacConkey Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J314	Cetrimide Selective Agar / MacConkey Agar / Vogel and Johnson Agar, 15x100mm Triplate, 7ml/section	10 plates/bag
Cat. no. J315	TSA Blood Agar / CLED Agar / MacConkey Agar, 15x100mm Triplate, 7ml/section	10 plates/bag

INTENDED USE

Hardy Diagnostics MacConkey Agar is recommended for use as a selective and differential medium for the isolation of gram-negative bacilli (including coliform organisms and enteric pathogens), on the basis of lactose fermentation.

SUMMARY

MacConkey Agar is a modification of Neutral Red Bile Salt Agar developed by MacConkey. It was one of the earliest culture media for the cultivation and identification of enteric organisms.⁽⁵⁾ It has also been used in the isolation of pathogens from foods and coliforms in water samples.^(8,9) The MacConkey Agar formulation presently in use is a modification of the original. In addition to containing sodium chloride, the modified formula has a lowered agar content and an adjusted concentration of bile salts and neutral red. Differentiation of enteric microorganisms is achieved by the combination of the neutral red indicator and lactose. Lactose-fermenting organisms form pink colonies surrounded by a zone of bile salt precipitation. Color change is due to the production of acid which changes the neutral red pH indicator from colorless to red. Acid production is also responsible for the formation of bile salt precipitation.

Non-lactose-fermenters (*Salmonella* spp. and *Shigella* spp.) develop into transparent, colorless colonies with no precipitated zone.

Peptones are incorporated into MacConkey Agar to provide amino acids and nitrogenous compounds. Sodium chloride is present to maintain osmotic equilibrium. Lactose is added as a possible carbon source for energy, and the acids produced from this activity precipitate out the bile salts. Bile salts and crystal violet are added to inhibit the growth of most gram-positive organisms.

FORMULA

Ingredients per liter of deionized water:*

Peptone	17.0gm
Lactose	10.0gm
Sodium Chloride	5.0gm
Proteose Peptone	3.0gm
Bile Salts	1.5gm
Neutral Red	30.0mg
Crystal Violet	1.0mg
Agar	13.5gm

Final pH 7.1 +/- 0.2 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light (Cat. no. Q63 may be stored at 2-30°C). Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious

disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.⁽¹⁻⁴⁾ Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation.

For MacConkey Agar deeps (Cat. no. Q63), liquify the medium by heating the number of desired tubes in a boiling water bath (100°C.). Cool the medium to 45-50°C. and aseptically pour the contents of each tube into separate sterile petri dishes. Allow the medium to solidify in plates for at least 30 minutes prior to use.

Method of Use: Allow plates to warm to room temperature. The agar surface should be dry before inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37°C. for 18-24 hours. Protect from light. Examine plates for colonial morphology.

INTERPRETATION OF RESULTS

Following incubation, the MacConkey Agar is examined for typical colonial morphology. Well isolated colonies of lactose-fermenting bacteria appear pink to red in color and are surrounded by a zone of bile salt precipitation. Non-lactose-fermenting colonies, such as *Shigella* spp. and *Salmonella* spp., appear transparent and colorless, with no zone of bile salt precipitation. Consult the listed references for further procedures for identification of isolates.⁽¹⁻⁴⁾

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The concentration of bile salts in MacConkey Agar is relatively low in comparison with other enteric plating media. The parallel use of more selective media for gram-negative enterics, such as HE (Cat. no. G63) or XLD (Cat. no. G65) is recommended in order to increase the chances of pathogen isolation.

Some strains of *Proteus* may swarm on this medium.

Serial inoculation may be required to assure adequate isolation of mixed flora samples.

It is recommended that the medium in agar deeps be melted only once prior to use. Do not reheat tubes multiple times. Discard any unused liquified agar deeps if not immediately poured into plates.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, slides, microscopes, staining supplies, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document

M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC® 25922	A	24hr	35°C	Aerobic	Growth; colonies pink to red with bile salt precipitate surrounding the colonies
<i>Proteus mirabilis</i> ATCC® 12453	A	24hr	35°C	Aerobic	Growth; colonies colorless with no swarming
<i>Salmonella enterica</i> ATCC® 14028	A	24hr	35°C	Aerobic	Growth; colonies colorless
<i>Enterococcus faecalis</i> ATCC® 29212	B	24hr	35°C	Aerobic	Partial to complete inhibition

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

MacConkey Agar should appear transparent, slightly opalescent, and pink in color.



Escherichia coli ATCC® 25922 colonies growing on MacConkey Agar. Incubated aerobically for 24 hours at 35°C.



Proteus mirabilis (ATCC® 12453) colonies growing on MacConkey Agar. Incubated aerobically for 24 hours at 35°C.



Salmonella enterica (ATCC® 14028) colonies growing on MacConkey Agar. Incubated aerobically for 24 hours at 35°C.



Enterococcus faecalis (ATCC® 29212) growth inhibited on MacConkey Agar. Incubated aerobically for 24 hours at 35°C.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. MacConkey, A.T. 1905. Lactose-fermenting bacteria in faeces. *J. Hyg.*; 5:333-379.
6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.
7. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
8. American Public Health Association. *Standard Methods for the Examination of Dairy Products*, APHA, Washington, D.C.
9. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

ATCC is a registered trademark of the American Type Culture Collection.

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[Ordering Information](#)

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