MACCONKEY AGAR + CRYSTAL VIOLET/ CHOCOLATE AGAR

INSTRUCTION FOR USE READY-TO-USE PLATED MEDIA

For professional use

Ref.:	Type of medium:	Packaging:
202112	ready-to-use medium-biplate	1x10 pcs (90 mm)

MACCONKEY AGAR + CRYSTAL VIOLET

Intended use: MacConkey Agar + Crystal Violet is used for the selective isolation and differentiation of Gram-negative enteric bacilli.

1. Principle: isolation and differentiation of Gram-negative enteric bacilli. Enzymatic digest of gelatin, enzymatic digest of casein, and enzymatic digest of animal tissue are the nitrogen and vitamin sources in MacConkey Agar + Crystal Violet. Lactose is the fermentable carbohydrate. During lactose fermentation a local pH drop around the colony causes a colour change in the pH indicator, neutral red, and bile precipitation. Bile salts mixture and crystal violet are the selective agents, inhibiting Gram-positive cocci and allowing Gram-negative organisms to grow. Sodium chloride maintains the osmotic environment. Agar is the solidifying agent.

2. Formula/Liter:

Enzymatic digest of gelatin	17.0 g
Enzymatic digest of casein	1.5 g
Enzymatic digest of animal tissue	1.5 g
Lactose	10.0 g
Bile salts mixture	1.5 g
Sodium chloride	5.0 g
Neutral red	0.03 g
Crystal violet	0.001 g
Agar	13.5 g

4. Appearance:

MACCONKEY AGAR + CRYSTAL VIOLET: prepared medium is clear and violet.

3. pH: 7.1 ± 0.2 at 25°C.

CHOCOLATE AGAR: prepared medium is brown and homogenous.

CHOCOLATE AGAR

Intended use: Chocolate Agar used for the isolation and cultivation of fastidious microorganisms.

1. Principle: enzymatic digest of casein and enzymatic digest of animal tissue provide nitrogen, carbon, and minerals. Corn starch absorbs any toxic metabolites produced. The phosphates are buffering agents. Sodium chloride maintains osmotic balance of the medium. Agar is the solidifying agent. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* spp. and enhanced growth of *Neisseria* spp. A chemical enrichment composed of cofactors, vitamins, and NAD (Biovitex) are also required for growth of *Haemophilus* spp. and *Neisseria* spp.

2. Formula/Liter:

Enzymatic digest of casein	7.5 g
Enzymatic digest of animal tissue	7.5 g
Corn starch	1.0 g
Agar	10.0 g
Sodium chloride	5.0 g
Monopotassium phosphate	1.0 g
Dipotassium phoshate	4.0 g
Growth supplement	2.0 ml
Hemoglobin solution (2%)	100 ml

3. pH: 7.2 ± 0.2 at 25°C.

5. Sample: clinical samples in which Gram-negative enteric bacilli and a wide variety of fastidious microorganisms including *Haemophilus influenzae* and *Neisseria* spp. are expected.

6. Test procedure: if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Streak the specimen for isolation onto the surface of the medium. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a sterile loop. Incubate plates aerobically at 35±2°C for 18- 24 hours in an inverted position.

7. Results: after incubation observe the growth of microorganism. Identification of the microorganism should be confirmed

by biochemical test.

8. Quality control: perform quality control testing for both negative and positive reaction by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. Graso uses following strains for performing quality control. Please note that other strains can be used in accordance with applicable local, state and laboratory's standard Quality Control.

MACCONKEY AGAR + CRYSTAL VIOLET:

Microorganism:	**Appearance of colony:**	**Recovery:**
Escherichia coli ATCC 25922	pink to rose-red	good growth (2)
Pseudomonas aeruginosa ATCC 10145	irregular, colourless to pink	good growth (2)
Proteus mirabilis ATCC 12453	colourless, swarming may be evident	good growth (2)
Salmonella typhimurium ATCC 14028	colourless	good growth (2)
Enterococcus faecalis ATCC 29212	-	no growth

CHOCOLATE AGAR WITH BIOVITEX:

Microorganism:	**Appearance of colony:**	**Recovery:**
Haemophilus influenzae ATCC 49766	small, mucoid, pearl	good growth (2)
Streptococcus pneumoniae ATCC 6305	small, shiny, flat with change of colour of the medium around colony	good growth (2)

9. Precautions: due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Although MacConkey Agar + Crystal Violet is a selective medium primarily for Gram-negative enteric bacilli, biochemical and serological testing using pure cultures are recommended for complete identification. Incubation of MacConkey Agar + Crystal Violet plates under increased CO_2 has been reported to reduce growth and recovery of a number of strains of Gram-negative bacilli with appropriate internal procedures and in accordance with local legislations. Although certain diagnostic tests may be performed directly on Chocolate Agar, biochemical and immunological testing using pure cultures are recommended for complete identification.

10. Disposal of waste: after use, all plates and any other contaminated materials must be sterilized or disposed of in line with appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

11. Storage: on receipt, store plates at 2-12°C away from direct sun light in an inverted position. Do not overload a refrigerator with excessive amounts of plates to avoid water condensation on the lids during storage. Plates must not come into direct contact with the inner walls of refrigerator, as the media may freeze, invalidating the tests. Prepared plates, stored in their original sleeve wrapping at 2-12°C until just prior to use, may be inoculated up to the expiration date and incubated for recommended incubation times. Plates from opened stacks of 10 plates should be used for two weeks when stored in a clean area at 2 to 12° C. Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or others signs of deterioration. Allow the medium to warm to the room temperature before inoculation.

All microbiological media containing dyes or light-sensitive components should be protected from light and stored in the dark.

Note that shelf life of the growth media changes after the addition of supplements. Complete media containing protein supplement tend to degrade faster than basal media alone.

12. Shelf life: 3 months.

13. Required supplements not supplied together with medium base: not applicable.

14. References: available on request.

Graso Zenon Sobiecki
Krąg 4A; 83-200 Starogard Gdański
www.grasobiotech.pl
tel. + 48 (58) 562 30 21

Production Department
Leśna 1, Owidz
83-211 Jabłowo

IVD