

GC AGAR (7104)

Intended Use

GC Agar is used with hemoglobin and enrichment for the isolation and cultivation of *Neisseria gonorrhoeae* and other fastidious organisms.

Product Summary and Explanation

In 1945, Johnston described a medium that successfully grew colonies of *N. gonorrhoeae* in 24 rather than 48 hours.¹ GC Agar was introduced in 1947 with reduced agar content. While investigating the growth rate of gonococcal strains, a medium containing growth factors glutamine and cocarboxylase was found to improve recovery.² In 1964, Thayer and Martin formulated a selective medium incorporating the antibiotics Polymyxin B and Rostocetin, with added supplements, into GC Agar.³ Thayer and Martin improved their medium by replacing the original antibiotics with a new microbial solution of Colistin, Vancomycin, and Nystatin (CVN).⁴ In 1970, Martin and Lester improved the new Thayer-Martin Medium by increasing the agar and glucose content and adding a new antibiotic, Trimethoprim Lactate (T).⁵ This improved medium was called Modified Thayer-Martin (MTM) Medium. Martin and Lewis improved selectivity of MTM by increasing the concentration of Vancomycin and replacing Nystatin with Anisomycin for greater inhibition of yeasts, known as Martin Lewis (ML) Agar.⁶ Transgrow Medium is a transport medium system incorporating either MTM or ML formulations.⁷

Principles of the Procedure

GC Agar is employed as a basal medium in the preparation of Chocolate Agar, Thayer-Martin Medium, Modified Thayer-Martin Medium, Martin-Lewis Agar, and Transgrow Agar.

Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide nitrogen, carbon, and minerals in GC Agar. 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. Chocolate Agar is prepared from GC Agar with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria* spp. A chemical enrichment composed of cofactors, vitamins, and nicotinamide adenine dinucleotide (NAD) are also required for growth of *Haemophilus* and *Neisseria* spp. If required, antimicrobial supplements are added as inhibitors for improved selectivity of the medium.

Formula / Liter

Enzymatic Digest of Casein	7.5 g
Enzymatic Digest of Animal Tissue.....	7.5 g
Corn Starch.....	1 g
Dipotassium Phosphate.....	4 g
Monopotassium Phosphate	1 g
Sodium Chloride	5 g
Agar	10 g

Final pH: 7.2 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplements

Hemoglobin Solution, 2%, 100 mL
Growth Enrichment, 2 mL
Antimicrobials, if required

Precaution

1. For Laboratory Use.

Directions, Double Strength

1. Suspend 7.2 g of the medium in 100 mL of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes. Cool to 45 – 50°C.
4. Prepare 100 mL of a 2% hemoglobin solution and autoclave at 121°C for 15 minutes.
5. Cool to 45 - 50°C and aseptically add to the molten GC Agar. Add 2 mL of growth enrichment. Add antimicrobials, if desired. Mix thoroughly and dispense.

Note: Refer to appropriate references for media formulations of Thayer-Martin Medium, Modified Thayer-Martin Medium, Martin Lewis Agar, and Transgrow Agar.^{8,9}

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared GC Agar supplemented as Chocolate Agar is opaque and brown.

Expected Cultural Response: Cultural response on Chocolate Agar at 35°C under CO₂ enrichment after 18 – 24 hours incubation.

Microorganism	Response
<i>Haemophilus influenzae</i> ATCC® 10211	growth
<i>Neisseria gonorrhoeae</i> ATCC® 43070	growth
<i>Neisseria meningitidis</i> ATCC® 13090	growth
<i>Streptococcus agalactiae</i> ATCC® 13813	growth
<i>Streptococcus pneumoniae</i> ATCC® 6303	growth

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

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* spp. and *Haemophilus* spp. consult procedures outlined in the references.^{8,9}

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

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

Limitation of the Procedure

Although certain diagnostic tests may be performed directly on GC Agar, biochemical and immunological testing using pure cultures are recommended for complete identification.

Packaging

GC Agar	Code No.	7104A	500 g
		7104B	2 kg
		7104C	10 kg

References

1. **Johnson, J.** 1945. Comparison of gonococcus cultures read at 24 and 48 hours. J. Venera. Dis. Inform. **26**:239.
2. **Lankford, C. E., V. Scott, M. F. Cox, and W. R. Cooke.** 1943. Some aspects of nutritional variation of the gonococcus. J. Bacteriol. **45**:321.
3. **Thayer, J. D., and J. E. Martin, Jr.** 1966. Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. Public Health Rep. **81**:559.
4. **Thayer, J. D., and A. Lester.** 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Service Rep. **86**:30.
5. **Martin, J. E., Jr., and R. L. Jackson.** 1975. A biological environmental chamber for the culture of *N. gonorrhoeae* with a new commercial medium. Public Health Rep. **82**:361.
6. **Martin, J. E., Jr., and J. S. Lewis.** 1977. Anisomycin: improved anti-mycotic activity in modified Thayer-Martin Medium. Public Health Rep. **35**:53.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.
8. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. vol. 1. American Society for Microbiology, Washington, D.C.
9. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.