Proteose No. 3 Agar

Intended Use

Proteose No. 3 Agar is used with added enrichment in isolating and cultivating Neisseria and Haemophilus.

Summary and Explanation

Proteose No. 3 Agar, introduced in 1938, is used for isolating Neisseria gonorrhoeae. When enriched with Hemoglobin and Supplement B,1,2 Proteose No. 3 Agar recovers gonococci in a manner comparable to more complex media, ranking only slightly lower than GC media at 24 hours.³

Chocolate agar may be prepared from Proteose No. 3 Agar with the addition of 2% Hemoglobin. Hemoglobin provides X factor (hemin), required for growth of Haemophilus and enhanced growth of Neisseria.

The growth rate of Neisseria and Haemophilus spp. may be improved with the addition of 1% Supplement B or VX, which provide the growth factors glutamine and cocarboxylase.

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen, vitamins and amino acids. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance in the medium, which is buffered by disodium phosphate. Agar is the solidifying agent.

Proteose Peptone No. 3 Agar is intended for use with supplementation by 2% Hemoglobin and Supplement B or Supplement VX.

Formula

Difco[™] Proteose No. 3 Agar

Approximate Formula* Per Liter	
Proteose Peptone No. 3	20.0
Dextrose	0.5
Sodium Chloride	5.0
Disodium Phosphate	5.0
Agar	15.0

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

Directions for Preparation from Dehydrated Product

- 1. Suspend 45.5 g of the powder in 500 mL 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. Cool to 50-60°C.
- 4. Aseptically add 500 mL sterile 2% solution of Hemoglobin and 10 mL of Supplement B or VX. Mix thoroughly.
- 5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For a complete discussion of the isolation and identification of Haemophilus or Neisseria spp., refer to the procedures outlined in the references.4-6

User Quality Control

Identity Specifications Difco[™] Proteose No. 3 Agar

Denydrated Appearance:	Beige, free-flowing, nomogeneous.
Solution:	9.1% (double strength) solution, soluble in puri- fied water upon boiling with frequent agitation. Solution is light to medium amber, opalescent with a slight flocculent precipitate.
Prepared Appearance:	(Single strength) light amber, opalescent with a slight flocculent precipitate.
Reaction of 9.1% Solution at 25°C:	рН 7.3 ± 0.2

Cultural Response Difco[™] Proteose No. 3 Agar

Prepare the medium per label directions (with 2% Hemoglobin and 1% Supplement B). Inoculate and incubate at $35 \pm 2^{\circ}$ C under 5-10% CO, for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Haemophilus influenzae	10211	10 ² -10 ³	Good
Neisseria gonorrhoeae	43070	10 ² -10 ³	Good
Neisseria meningitidis	13102	10 ² -10 ³	Good
Neisseria sicca	9913	10 ² -10 ³	Good

Expected Results

Refer to appropriate references and procedures for results.

Limitation of the Procedure

Proteose No. 3 Agar is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.

References

g

g g

g

g

- Lankford, Scott, Cox and Cooke. 1943. J. Bacteriol. 45:321. Lankford and Snell. 1943. J. Bacteriol. 45:410.

- Lankford and Snell. 1943. J. Bacteriol. 45:410.
 Carpenter, Bucca, Buck, Casman, Christensen, Crowe, Drew, Hill, Lankford, Morton, Peizer, Shaw and Thayer. 1949. Am. J. Syphil. Gonorrh. Vener. Dis. 33:164.
 Isenberg and Garcia (ed.). 2004 (update, 2007). Clinical microbiology procedures handbook, 2nd ed. American Society for Microbiology, Washington, D.C.
 Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
 Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Jouis Mo.
- St. Louis, Mo.



Availability

Difco[™] Proteose No. 3 Agar Cat. No. 265100 Dehydrated – 500 g

BBL[™] Hemoglobin Solution 2% Cat. No. 211874 Bottle – 10 × 100 mL*

BBL[™] Hemoglobin, Bovine, Freeze-Dried

Cat. No. 212392 Dehydrated – 500 g

Difco[™] Supplement B

Cat. No. 227610 Lyophilized – 6 × 10 mL with Reconstituting Fluid* 227620 Lyophilized – 100 mL with Reconstituting Fluid*

Difco[™] Supplement VX

*Store at 2-8°C.

Cat. No. 233541 Lyophilized – 6×10 mL with Reconstituting Fluid* 233542 Lyophilized – 100 mL with Reconstituting Fluid*

