COLUMBIA AGAR (7734)

Intended Use

Columbia Agar is used with or without blood for the isolation and cultivation of a wide variety of fastidious microorganisms. Conforms to Harmonized USP/EP/JP Requirements.[1,2,3]

Product Summary and Explanation

Columbia Agar is a nutritiously rich general purpose medium developed by Ellner et al.[4] from Columbia University. This medium contains a mixture of peptones, which provides a blend of nitrogenous compounds and amino acids to enhance growth. Columbia Agar is used for the cultivation of fastidious microorganisms and as a base for the preparation of various specialized culture media to which supplements can be added. Columbia Agar can be supplemented with 5 - 10% sheep, rabbit, or horse blood for use in isolating, cultivating, and determining hemolytic reactions of fastidious pathogenic microorganisms.

The addition of antibiotics, kanamycin or gentamicin, to Columbia Agar suppresses the growth of certain Gram-negative and Gram-positive microorganisms. Columbia Agar can provide an anaerobic environment when supplemented with growth factors, hemin, vitamin K_1, and sheep blood.

Columbia Agar conforms to Harmonized United States Pharmacopoeia (USP), European Pharmacopoeia (EU), and Japanese Pharmacopoeia (JP).[1,2,3]

Principles of the Procedure

The nitrogen, vitamin, and amino acids are provided by a combination of peptones including, Pancreatic Digest of Casein, Meat Peptic Digest, and Heart Pancreatic Digest. Yeast Extract and Maize Starch are included to supply a carbon energy source in the form of B-complex vitamins. Sodium Chloride maintains the osmotic balance of the medium. Agar is the solidifying agent.

Supplementation with blood (5 - 10%) provides additional growth factors for fastidious microorganisms, and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[5] In general, blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of β-hemolytic streptococci.[6]

Formula / Liter

Pancreatic Digest of Casein	10 g
Meat Peptic Digest	5 g
Heart Pancreatic Digest	3 g
Yeast Extract	5 g
Maize Starch	1 g
Sodium Chloride	5 g
Agar	12 g

Final pH: 7.3 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to skin, eyes, and mucous membranes

Directions

1. Dissolve 41 g of the medium in one liter 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.

Quality Control Specifications

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

Prepared Appearance: Prepared medium without blood is trace to very slightly hazy and light amber. With 5% sheep blood the medium is opaque and red.

Expected Cultural Response and USP/EP/JP Growth Promotion Testing: Columbia Agar was prepared according to label directions and inoculated with the organisms listed below. Cultures were incubated under appropriate atmosphere at 30 - 35°C and examined for growth at 18 – 48 hours.

Without 5% defibrinated sheep blood:

Microorganism	Approx. Inoculum (CFU)	Expected Results
Escherichia coli ATCC® 25922	10 - 100	Growth
Pseudomonas aeruginosa ATCC® 9027	10 - 100	Growth
Staph aureus ATCC® 25923	10 - 100	Growth
Streptococcus pyogenes ATCC® 19615	10 - 100	Growth

With 5% defibrinated sheep blood:

Escherichia coli ATCC® 25922	10 - 100	Growth
Listeria monocytogenes ATCC® 7644	10 - 100	Growth
Staphylococcus aureus ATCC® 25923	10 - 100	Growth
Streptococcus pneumoniae ATCC® 6305	10 - 100	Growth
Streptococcus pyogenes ATCC® 19615	10 - 100	Growth

This culture was tested at Harmonized USP/EP/JP specified temperatures and incubation times.[1,2,3]

Clostridium sporogenes coli ATCC® 11437	10 - 100	Growth

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

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with inoculating loop.
2. If supplemented with blood, stab agar several times to deposit beta-hemolytic streptococci beneath agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[5]
3. Incubate plates aerobically, anaerobically, or under conditions of increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Results

Examine Columbia Agar for growth.

For Columbia Agar, supplemented with blood, examine the medium for growth and hemolytic reactions after 18 - 24 and 48 hours incubation. There are four types of hemolysis on blood agar media described as:[7]

1. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This produces a green discoloration of the medium.
2. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
3. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
4. Alpha-prime-hemolysis (α') is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

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.

Limitations of the Procedure

1. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human, and rabbit blood agar and alpha-hemolytic on sheep blood agar.[5]
4. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[5] For optimal performance, incubate blood agar base media under increased CO_2 (5 - 10%) in accordance with established laboratory procedures.

Packaging

Columbia Agar	**Code No.**	**7734A**	**500 g**
		7734B	**2 kg**
		7734C	**10 kg**

References

1. **United States Pharmacopeial Convention.** 2007. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.
2. **Directorate for the Quality of Medicines of the Council of Europe (EDQM).** 2007. The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France.
3. **Japanese Pharmacopoeia.** 2007. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare.
4. **Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi.** 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. **45**:502-504.
5. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
6. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
7. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, Clinical microbiology procedures handbook, vol. 1 p. 1.61-1.67. 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 (517)372-9200 or fax us at (517)372-2006.

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