

## 27688 Columbia Agar (Columbia Blood Agar)

This high-grade complete medium acc. to Ellner is for the cultivation of fastidious microorganisms. It is also used as a base for the preparation of a variety of special media. The addition of blood permits the interpretation of hemolytic activities.

### Composition:

Ingredients	Grams/Litre
Special nutrient substrate	23.0
Starch	1.0
Sodium chloride	5.0
Agar	15.0
Final pH 7.3 +/- 0.2 at 25°C.	

Store prepared media below 8°C, protected from direct light. Prepared media should not be used if there are signs of contamination or deterioration (shrinking, cracking, evaporation or discoloration). Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

### Directions:

Dissolve 42 g in 1 litre distilled water. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C and add heat sensitive components. Incubate at 35°C under anaerobic conditions.

*Columbia Agar with 5% blood:* Add 5% blood to Columbia Agar, mix well and pour plates.

*Boiled Blood Agar ("Chocolate Agar"):* Is prepared by heating after 10% sterile defibrinated blood has been added to the sterilized Columbia Agar (10 min at 80°C). The medium becomes chocolate brown in colour, then pour plates.

*Gentamicin Blood Agar:* Add 10% blood to Columbia Agar, mix well and supplement 5 mg/l gentamycin (e.g. 0.1 ml gentamycin solution Cat. No. 48755). Pour plates.

*Colombia CNA Agar:* Add to the 42 g dehydrated medium 10 mg Colistin sulfate and 15 mg Nalidixic acid and solve in 1 litre distilled water. Sterilize by autoclaving at 121°C for 15 minutes.

*Lactose Milk Egg-Yolk Agar:* Add to the 42 g dehydrated medium 12 g Lactose (Cat. No. 61345) and 1 g Agar (e.g. Cat. No. 05040). Solve in 1 litre distilled water. Mix in 33 ml of a 0.1 % aqueous solution of neutral red (Cat. No. 72210), adjust the pH to 7.0 and autoclave (15 min at 121 °C). Cool to 45-50°C, add about 35 ml Egg-Yolk emulsion (Cat. No. 17148) and 10 g Skim Milk powder (Cat. No. 70166) and mix well. Pour plates.

*Columbia Agar for Campylobacter:*

Rehydrate the contents of one vial Campylobacter Supplement III (Skirrow, Cat. No. C3222) with 2 ml of sterile distilled water and mix well to dissolve. Avoid frothing of the solution. Aseptically add the contents of the vial to 500 ml of sterile, molten Columbia Agar with 5-7% v/v lysed defibrinated horse or sheep blood. Mix gently and pour into sterile petri plates.



## Principle and Interpretation:

The Columbia Agar can also be utilized as a base to prepare a wide range of other media by adding special supplement for selective cultivation. Chocolate agar supplemented with 10% blood is suited for isolating *Haemophilus* and *Neisseria* species. Gentamicin Blood Agar is for the selective cultivation of *Streptococcus pneumoniae* and other Streptococci as well as bacterioides, *Clostridium* and yeasts. The Columbia CNA Agar suppresses the growth of *Proteus*, *Klebsiella* and *Pseudomonas* species while Staphylococci, haemolytic Streptococci and Enterococci still growing. Lactose Milk Egg-Yolk Agar is recommended for the isolation of fastidious *Clostridia*. The addition of blood, cycloserine and cefoxitin to Columbia Agar is recommended for the isolation of *Clostridium difficile*. It can also be employed in the so-called *Corynebacterium diphtheriae* toxicity (virulence) test when using the agar plate diffusion method. It is used to prepare Vaginalis agar for the cultivation of *Gardnerella vaginalis*. Also possible is to make Acriflavin-Ceftacidim Agar (AC Agar) for the selective cultivation of *Listeria* from foodstuffs.

Cultural characteristics from Columbia Agar after 24-48 hours at about 35°C.

Organisms (ATCC)	Growth without blood	Growth with blood	Hemolysis
<i>Streptococcus pyogenes</i> (19615)	+++	+++	beta
<i>Streptococcus pneumoniae</i> (6305)	+++	+++	alpha
<i>Escherichia coli</i> (25922)	+++	+++	-
<i>Staphylococcus aureus</i> (25923)	+++	+++	beta

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### **Precautions and Disclaimer**

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