

## COLUMBIA AGAR BASE (EUROPEAN PHARMACOPOEIA/ ISO 10272)

**CAT N°: 1104**

For the isolation and cultivation of fastidious microorganisms and the determination of hemolytic reactions

### FORMULA IN g/l

Pancreatic Digest of Casein	10.00	Heart Pancreatic Digest	3.00
Yeast Extract	5.00	Maize Starch	1.00
Meat Peptic Digest	5.00	Bacteriological Agar	13.50
Sodium Chloride	5.00		

**Final pH 7.3 ± 0.2 at 25°C**

### PREPARATION

Suspend 42.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. The medium is generally enriched with sterile defibrinated blood (sheep, rabbit or horse), serum or some other material. Cool to 45 - 50°C and aseptically add 5 - 10% sterile defibrinated blood, homogenize gently and pour into Petri dishes. Be careful to avoid bubble formation when adding the blood. The prepared medium should be stored at 8-15°C. The color of the prepared medium without blood is clear amber, slightly opalescent. The color of the prepared medium with blood is cherry red opaque.

The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium.

To make the medium more selective, the following supplements may be added if desired:

CNA Staph/Streph Supplement (Cat. 6016) for staphylococci, streptococci and pneumococci isolation

Brucella Supplement (Cat. 6017) for *Brucella* isolation

### USES

COLUMBIA AGAR BASE is a highly nutritive general purpose medium for the cultivation of fastidious organisms, especially when used as a base for Blood Chocolate Agar. It can also be used as a selective isolation medium by adding antimicrobial agents. Columbia Agar Base is used extensively as a medium base for a variety of culture formulations in medical bacteriology. The hemolytic reactions in blood agar are genuinely defined. The majority of the common pathogenic bacteria, however, grow well without the addition of blood.

Pancreatic digest of casein, Meat peptic digest and Heart pancreatic digest provide nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract and Maize starch are source of vitamins, particularly of the B-group essential for bacterial growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent. The blood is another source providing growth factors for the microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary according to the type of blood or base medium used. For example, defibrinated sheep blood gives best results for Group A streptococci.

The European Pharmacopoeia recommends in the Paragraph 2.6.13 "Microbiological examination of non-Sterile products: test for specified microorganisms" that the Reinforced Clostridial Medium (Cat. 1007) should be incubated under anaerobic conditions at 30-35°C for 48 hours. After incubation, make subcultures from each tube on Columbia Agar and incubate under anaerobic conditions at 30-35°C for 48 hours. The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This should be confirmed by identifications tests.

If no anaerobic growth of microorganisms is detected on Columbia agar or the catalase test is positive, the product complies with the test.

With the addition of 5 - 10% sterile defibrinated blood and, especially with Polyenrichment Supplement (Cat. 6011) and VCN Supplement (Cat. 6013), whilst the patient is receiving antibiotic treatment, Columbia Agar Base becomes an excellent chocolate agar that can be used to isolate pathogenic *Neisseria*, gonococci and meningococci, as good as or better than Thayer-Martin Medium.

Depending on the selection of inhibitors, alternative supplements to VCN may be VCNT (Cat. 6026) or VCAT (Cat. 6014).

Inoculate and incubate at 30-35, under 5-10% CO<sub>2</sub> during 48 hours.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium, with 5% sterile defibrinated sheep blood added, from type cultures after incubation at a temperature of 30-35°C, under 5-10% CO<sub>2</sub>, and observed after 48 hours.

Microorganisms	Growth	Hemolysis
<i>Neisseria meningitidis</i> ATCC 13090	Good	-----
<i>Staphylococcus aureus</i> ATCC 25923	Good	Beta/Gamma
<i>Streptococcus pneumoniae</i> ATCC 6303	Good	Alpha
<i>Streptococcus pyogenes</i> ATCC 19615	Good	Beta
* <i>Clostridium sporogenes</i> ATCC 11437	Good	

\*According European Pharmacopoeia incubate at 30-35 °C under 5-10% CO<sub>2</sub> for 48-72 hours.

## BIBLIOGRAPHY

Ellner, Stossel, Drakeford and Vasi. AM J. Clin. Path. 45:502-504. 1966.

European Pharmacopoeia. 7.0



## STORAGE

Once opened keep powdered medium closed to avoid hydration.

