

# COLUMBIA AGAR WITH 5% SHEEP BLOOD

## INSTRUCTION FOR USE READY-TO-USE PLATED MEDIA

For professional use

**Intended use:** Columbia Agar with 5% sheep blood is used for the isolation and cultivation of a wide variety of fastidious microorganisms.

Ref.:	Type of medium:	Packaging:
201190	ready-to-use medium-plate	1x10 pcs (90 mm)

**1. Principle:** a mixture of peptones including enzymatic digest of animal tissue, enzymatic digest of casein, and yeast enriched peptone provides a good source of nitrogen, carbon and other nutrients to microbiological cultures. Corn starch increases the growth of *Neisseria* spp., and enhances the haemolytic reactions of some streptococci. 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 enables haemolysis determination. Haemolytic patterns may vary with the source of animal blood and the type of basal medium used.

### 2. Formula/Liter:

Enzymatic digest of casein	5.0 g
Enzymatic digest of animal tissue	8.0 g
Yeast enriched peptone	10.0 g
Agar	14.0 g
Sodium chloride	5.0 g
Corn starch	1.0 g

### Supplements/Liter:

Sheep blood 50 ml

**3. pH:** 7.3 ± 0.2 at 25°C.

### 4. Appearance:

**Prepared Appearance:** prepared medium without blood is light straw to straw. With 5% sheep blood the medium is red.

**5. Sample:** all samples in which a wide variety of fastidious microorganisms is expected. Application: A multi-purpose medium suitable for the cultivation of a wide variety of fastidious microorganisms.

**6. Test procedure:** if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Streak the specimen for isolation onto the surface of the medium. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a sterile loop. Incubate plates aerobically at 35±2°C for 18 - 24 hours in an inverted position.

**7. Results:** after incubation observe growth and haemolysis characteristic for a particular microorganism. Identification of the microorganism should be confirmed by biochemical test.

**8. Quality control:** perform quality control testing by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. Graso uses following strains for performing quality control. Please note that other strains can be used in accordance with applicable local, state and laboratory's standard Quality Control.

Microorganism:	Appearance of colony:	Haemolysis:
<i>Escherichia coli</i> ATCC 25922	large, flat, gray, smooth, shiny	—
<i>Pseudomonas aeruginosa</i> ATCC 10145	large, flat, irregular edge, gray, opaque	—
<i>Staphylococcus aureus</i> ATCC 25923	circular, white to cream, entire edge, smooth, convex	type β
<i>Enterococcus faecalis</i> ATCC 29212	small, circular, entire edge	type γ
<i>Streptococcus pyogenes</i> ATCC 19615	very small, white to gray with clear zone of hemolysis around colony	type β
<i>Streptococcus pneumoniae</i> ATCC 6305	very small, flat, entire edge	type α

**9. Precautions:** due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Haemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-haemolytic on horse, human, and rabbit blood agar and alpha-haemolytic on sheep blood agar. Atmosphere of incubation has been shown to influence haemolytic reactions of beta-haemolytic streptococci. For optimal performance, incubate blood agar base media under increased CO<sub>2</sub> (5 - 10%) in accordance with the established laboratory procedures.

**10. Disposal of waste:** after use, all plates and any other contaminated materials must be sterilized or disposed of in line with appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

**11. Storage:** On receipt, store plates at 2-12°C away from direct sun light in an inverted position. Do not overload a refrigerator with excessive amounts of plates to avoid water condensation on the lids during storage. Plates must not come into direct contact with the inner walls of refrigerator, as the media may freeze, invalidating the tests. Prepared plates, stored in their original sleeve wrapping at 2-12°C until just prior to use, may be inoculated up to the expiration date and incubated for recommended incubation times. Plates from opened stacks of 10 plates should be used for two weeks when stored in a clean area at 2 to 12° C. Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or others signs of deterioration. Allow the medium to warm to the room temperature before inoculation.

All microbiological media containing dyes or light-sensitive components should be protected from light and stored in the dark.

Note that shelf life of the growth media changes after the addition of supplements. Complete media containing protein supplement tend to degrade faster than basal media alone.

**12. Shelf life:** 55 days.

**13. Required supplements not supplied together with medium base:** not applicable.

**14. References:** available on request.



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