

# MUELLER HINTON II AGAR WITH 5% SHEEP BLOOD

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

For professional use

*Intended use: Mueller Hinton II Agar with 5% sheep blood is used specifically for the susceptibility testing of a wide variety of fastidious microorganisms by the disk diffusion method. This formula conforms to CLSI.*

Ref.	Type of medium:	Packaging:
1172	ready-to-use medium-plate	1x10 pcs (90 mm)

**1. Principle:** beef extract and casein peptone provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar with sheep blood. Corn starch is added to absorb any toxic metabolites produced. Defibrinated sheep blood enables the growth of fastidious bacteria. Agar is the solidifying agent.

### 2. Formula/Liter:

Casein peptone	17.5 g
Corn starch	1.5 g
Beef extract	2.0 g
Agar	17.0 g

### Supplements / Liter:

Sheep blood 50.0 ml

**3. pH:**  $7.3 \pm 0.1$  at 25°C.

### 4. Appearance:

**Prepared Appearance:** prepared medium is homogenous and red.

**5. Sample:** all fastidious microorganisms dedicated to susceptibility testing by the disk diffusion method .

**6. Test procedure:** if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Prepare of 0,5 McFarland suspension of tested microorganism. Within 15 minutes, dip a sterile swab into the suspension, squeeze it against the walls of the tube to remove excess liquid, then streak it over the surface of the agar plates to obtain a uniform distribution of the inoculum. Leave the plates to dry then lay the paper discs pressing them onto the surface of the agar. Incubate at  $35 \pm 2^\circ\text{C}$  for 24 h then read the inhibition zones by taking in to consideration the zones, which are completely free of microbial growth and which have distinct borders.

**7. Results:** after incubation time read the zones around antibiotic discs. Compare the zone sizes obtained to those reported on the tables of the CLSI.

**8. Quality control:** perform quality control for the susceptibility testing of *Streptococcus pneumoniae* by the disk diffusion method according to CLSI.

Microorganism:	Growth:
<i>Streptococcus pneumoniae</i> ATCC 49619	good growth
<i>Streptococcus pyogenes</i> ATCC 19615	good growth

**9. Precautions:** numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH. Strict adherence to protocol is required to ensure reliable results. Drug inactivation may result from the prolonged incubation times required by slow growers.

**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  $121^\circ\text{C}$  for at least 20 minutes.

**11. Storage:** On receipt, store plates at 6-12°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 6-12°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 6 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.

**12. Shelf life:** 45 days.

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

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



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