MUELLER HINTON II AGAR ACCORDING TO EUCAST

INSTRUCTION FOR USE

For professional use

Intended use: Mueller Hinton II Agar is used specifically for the susceptibility testing of bacteria by the disc and for determining the Minimum Inhibitory Concentration (MIC) diffusion method. This formula conforms to EUCAST.

Ref.:	Type of medium:	Packaging:
197	dehydrated medium	500 g
3096	medium in bottle	100, 200, 500 ml
1051	ready-to-use medium-plate	1x10 pcs (90 mm)
4003A	ready-to-use bi-plate medium	1x5 pcs (120 mm)
4006	ready-to-use bi-plate medium	1x5 pcs (140 mm)

1. Principle: beef extract and acid hydrolysate of casein provide nitrogen, vitamins, carbon, and amino acids. Corn starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent.

2. Formula/Liter:

Acid hydrolysate of casein	17.5 g
Corn starch	1.5 g
Beef extract	2.0 g
Agar	17.0 g
ions Mg^{++}	20-35 mg/l
ions Ca^{++}	45-75 mg/l

Supplements/Liter:

3. pH: 7.3 ± 0.1 at 25°C.

4. Preparation:

Dehydrated medium:
Suspend 38.0 g of the medium in one liter of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes. Cool to 45 - 50°C, mix well. Pour into sterile Petri dishes.*

Bottles with agar :
Agar in the bottles should be melted in water batch at 80ºC or microwaved.

Melting of agar base in microwave
1. Loosen the cap on the agar bottle before microwaving.
2. Place the bottle with agar base in central place of microwave.
3. Heat in one-minute intervals on low power until all of the agar is melted.
4. Between intervals, gently swirl the bottle to make sure the agar is melting evenly.
5. While wearing heat-protective gloves, carefully remove the hot bottle and let it cool to 45-50°C.
6. Mix well and pour on Petri dishes.*

Melting of agar base in water bath
1. Loosen the cap on the agar bottle and place it into the water bath.
2. Keep the water temperature at around 80°C.
3. Leave the bottle with the agar base in the water bath until the agar is completely melted.
4. While wearing heat-protective gloves, carefully remove the hot bottle, stir it gently and let it cool to 45-50°C.
5. Mix well and pour on Petri dishes.*

** Working in a clean, draft-free area disinfected with bactericidal cleaner. Take care to use aseptic techniques to prevent contamination. Working on one plate at a time, carefully tilt open the cover and pour about 15–20 ml of liquid into the bottom portion (it should cover about 2/3 of the plate's surface). Gently rotate the dish to ensure that the liquid medium covers the base of the dish evenly. The layer should be about 3–4 mm deep. Allow plates to solidify and cool before use. This takes about one hour. Do not put agar plates in a freezer to speed up this process. Dry the plates with the lid slightly off for 20 minutes in the laminar flow hood or a 37°C incubator to avoid water evaporation and condensation on the lid during storage or incubation.*

5. **Appearance:**

Dehydrated Appearance: dehydrated medium is homogeneous, free flowing, beige.

Prepared Appearance: prepared medium is clear and straw.

6. Sample: all microorganisms dedicated to susceptibility testing by the disk diffusion method and by MIC tests method.

7. Test procedure: *disc diffusion method*: 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 ± 1°C for 18 ± 2h hours then read the inhibition zones by taking in to consideration the zones, which are completely free of microbial growth and which have distinct borders. *MIC test method*: 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 apply the strip to the agar surface with the MIC scale facing upwards and code ofthe strip to the outside of the plate, pressing it with a sterile forceps on the surface of the agar and ensure that whole length of the antibiotic gradient is in complete contact with the agar surface. Once applied, do not move the strip. Incubate plates in an inverted position at 35 ± 1°C for 18 ± 2h hours.

8. Results: *disc diffusion method*: after incubation time read the zones around antibiotic discs. Compare the zone sizes obtained to those reported on the tables of the EUCAST. *MIC test method*: after incubation read the MIC value where the edge of the inhibition ellipse intersects the strip (intersection between two scale segments should be round up to the higher value). Compare the result obtained to those reported on the tables of the EUCAST.

9. Quality control: perform quality control for the susceptibility testing according to EUCAST.

Microorganism:	**Appearance of colony:**	**Growth:**
Escherichia coli ATCC 25922	large, light straw	good growth (2)
Staphylococcus aureus ATCC 29213	medium to large, circular, cream, convex	good growth (2)
Pseudomonas aeruginosa ATCC 27853	large, flat, irregular edge, shiny	good growth (2)
Enterococus faecalis ATCC 29212	small,circular, enitre edge	good growth (2)

10. 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. Variation in the concentration of divalent cations, primarily calcium and magnesium affects result of aminoglycoside, tetracycline, and colistin test with *P. aeruginosa* isolates

11. 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.

12. Storage: sealed, unopened containers with dehydrated powdered media should be stored at 2-30°C. Once opened and recapped, place the container in a low humidity environment at room temperature. Protect from moisture and light. Bottles should be stored at 6-25°C in the dark used before the expiry date on the label. 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.

13. Shelf life: dehydrated medium: 3 years,
bottles: 1 year,
plates: 3 months.

14. Required supplements not supplied together with medium base: not applicable.

15. References: available on request.

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