

## TM 339 -MUELLER HINTON AGAR

### INTENDED USE

For cultivation of *Neisseria* spp. & for determination of susceptibility of microorganisms to antimicrobial agents isolated from clinical samples.

### PRODUCT SUMMARY AND EXPLANATION

The Mueller Hinton formulation was originally developed as a simple, transparent agar medium for the cultivation of pathogenic *Neisseria* species. Other media were subsequently developed that replaced the use of Mueller Hinton Agar for the cultivation of pathogenic *Neisseria* species, but it became widely used in the determination of sulfonamide resistance of gonococci and other organisms. Mueller Hinton Agar is now used as a test medium for antimicrobial susceptibility testing. Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gel as described in CLSI Approved Standard. Mueller Hinton Agar has been selected by the CLSI for several reasons:

- i. It demonstrates good batch-to-batch reproducibility for susceptible testing.
- ii. It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
- iii. It supports the growth of most non-fastidious bacterial pathogens.
- iv. Many data and much experience regarding its performance have been recorded.

Kirby-Bauer et al recommended this medium for performing antibiotic susceptibility tests using a single disc of high concentration. WHO Committee on Standardization of Susceptibility Testing has accepted Mueller Hinton Agar for determining the susceptibility of microorganisms because of its reproducibility. Mueller Hinton Agar with 5% sheep blood and Mueller Hinton Agar with Hemoglobin have been recommended for antimicrobial susceptibility testing of *Streptococcus pneumoniae* and *Haemophilus influenzae*.

The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values. A standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standards. The various factors, which influence disc diffusion susceptibility tests, are agar depth, disc potency, inoculum concentration, pH of the medium and beta-lactamase production by test organisms.

Mueller Hinton Agar is not appropriate for assay by disc diffusion method with slow growing organisms, anaerobes and capnophiles. With slow growing organisms, increased incubation may cause deterioration of diffusing antibiotic and produce unprecise readings.

### COMPOSITION

Ingredients	Gms / Ltr
Beef extract	2.000
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

### PRINCIPLE

Beef extract and Casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch acts as a protective colloid against toxic substances present in the medium. Starch hydrolysis yields dextrose, which serves as a source of energy. These ingredients are selected for low thymine and thymidine content as determined by MIC values for *Enterococcus faecalis* with sulfamethoxazole trimethoprim (SXT).



**INSTRUCTION FOR USE**

- Dissolve 38.0 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Note: The performance of this batch has been tested and standardised as per the current CLSI (formerly, NCCLS) document M6-protocols for Evaluating Dehydrated Mueller Hinton Agar.

**QUALITY CONTROL SPECIFICATIONS**

**Appearance of Powder** : Cream to yellow homogeneous free flowing powder.

**Appearance of prepared medium** : Light amber coloured clear to slight opalescent gel forms in Petri plates.

**pH (at 25°C)** : 7.3±0.2

**INTERPRETATION**

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Zone of inhibition Observed	Standard Zone	Incubation Temperature	Incubation Period
<i>Escherichia coli</i>	25922	50-100	luxuriant	≥70%	-	-	30-35°C	18 -24 Hours
<i>Cephalothin CEP</i> 30mcg	-	-	-	-	29-37 mm	29 -37 mm	-	-
<i>Chloramphenicol C</i> 30 mcg	-	-	-	-	21-27 mm	21 -27 mm	-	-
<i>Co-Trimoxazole COT</i> 25	-	-	-	-	23-29 mm	23 -29 mm	-	-
<i>Cefotaxime CTX</i> 30 mcg	-	-	-	-	29-35 mm	29 -35 mm	-	-
<i>Gentamicin GEN</i> 10 mcg	-	-	-	-	19-26 mm	19 -26 mm	-	-
<i>Sulphafurazole SF</i> 300 mcg	-	-	-	-	15-23 mm	15 -23 mm	-	-
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	25923	50-100	luxuriant	≥70%	-	-	30-35°C	18 -24 Hours
<i>Co-Trimoxazole COT</i> 25 <i>Thymidine Content</i>	-	-	-	-	20 mm (Clear zone)	≥20 mm	-	-
<i>Cefoxitin CX</i> 30 mcg	-	-	-	-	23-29 mm	23 -29 mm	-	-
<i>Erythromycin E</i> 15 mcg	-	-	-	-	22-30 mm	22 -30 mm	-	-
<i>Linezolid LZ</i> 30 mcg	-	-	-	-	25-32 mm	25 -32 mm	-	-



<i>Oxacillin OX</i> 1mcg	-	-	-	-	18-24 mm	18 -24 mm	-	-
<i>Pristinomycin RP</i> 15 mcg	-	-	-	-	21-28 mm	21 -28 mm	-	-
<i>Tetracycline TE</i> 30 mcg divalent	-	-	-	-	18-25 mm	18 -25 mm	-	-
<i>Ciprofloxacin CIP</i> 5mcg	-	-	-	-	22-30 mm	22 -30 mm	-	-
<i>Pseudomonas aeruginosa</i>	27853	50-100	luxuriant	>=70%	-	-	30-35°C	18 -24 Hours
<i>Ceftazidime CAZ</i> 30 mcg	-	-	-	-	22-29 mm	22 -29 mm	-	-
<i>Ciprofloxacin CIP</i> 5mcg	-	-	-	-	30-40 mm	30 -40 mm	-	-
<i>Tobramycin TOB</i> 10 mcg divalent	-	-	-	-	19-25 mm	19 -25 mm	-	-
<i>Amikacin AK 30</i> mcg divalent	-	-	-	-	18-26 mm	18 -26 mm	-	-
<i>Aztreonam AT</i> 3mcg	-	-	-	-	23-29 mm	23 -29 mm	-	-
<i>Cephotaxime CTX</i> 30 mcg	-	-	-	-	18-22 mm	18 -22 mm	-	-
<i>Gentamicin GEN</i> 10 mcg divalent	-	-	-	-	16-21 mm	16 -21 mm	-	-
<i>Imipenem IPM</i> 10 mcg	-	-	-	-	20-28 mm	20 -28 mm	-	-
<i>Piperacillin PI</i> 100 mcg	-	-	-	-	12-18 mm	25 -33 mm	-	-
<i>Escherichia coli</i>	35218	50-100	luxuriant	>=70%	-	-	30-35°C	18 -24 Hours
<i>Amoxyclav AMC</i> 30 mcg	-	-	-	-	18-24 mm	18 -24 mm	-	-
<i>Piperacillin/Tazo</i> <i>bactam</i> PIT100/10 mcg	-	-	-	-	24-30 mm	24 -30 mm	-	-
<i>Ticarillin TI</i> 75 mcg	-	-	-	-	6 mm	6 -6 mm	-	-
<i>Ticarillin/Clavul</i> <i>anic acid TCC</i> 75/10mcg	-	-	-	-	20-28 mm	20 -28 mm	-	-
<i>Ampicillin AMP</i> 10 mcg	-	-	-	-	16-22 mm	16 -22 mm	-	-
<i>Ampicillin/Sulba</i> <i>ctam A/S10/10</i> mcg	-	-	-	-	29-37 mm	29 -37 mm	-	-



<i>Enterococcus faecalis</i>	29212	50-100	luxuriant	>=70%	-	-	30-35°C	18 -24 Hours
<i>Trimethoprim TR 5 mcg Thymine</i>	-	-	-	-	20 mm	>=20 mm	-	-
<i>Vancomycin VA 30 mcg</i>	-	-	-	-	17-21 mm	17 -21 mm	-	-
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	43300	50-100	luxuriant	>=70%	-	-	30-35°C	18 -24 Hours
<i>Oxacillin OX 1 mcg</i>	-	-	-	-	very hazy to no zone	No zone	-	-

**PACKAGING:**

In pack size of 100 gm and 500 gm bottles.

**STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 10-25°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.




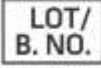



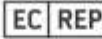




**DISPOSAL**

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

**REFERENCES**

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 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Buckenhof 10 48143 Aachen, Germany</small>	 European Conformity	 QR Code	 Consults instructions for Use	 Best Before

**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**  
**Revision: 08 May., 2023**