

## TRM 405 -C.L.E.D. AGAR (W/ BROMO THYMOL BLUE) (BROLACIN AGAR)

### INTENDED USE

For isolation and differentiation of urinary pathogen by lactose fermentation.

### PRODUCT SUMMARY AND EXPLANATION

CLED Agar (Cystine Lactose- Electrolyte- Deficient Agar) w/ Bromo Thymol Blue is a differential medium that was described by Mackey and Sandys to isolate coliforms from urine samples. On a solid medium, Sandys reported that swarming of *Proteus* species can be controlled by restricting the electrolytes. Later on Sandys medium was modified by Mackey and Sandys, by replacing mannitol with lactose and sucrose and elevating the concentration of agar and bromo thymol blue. This formulation was further modified by the same authors, called C.L.E.D. (Cystine-Lactose-Electrolyte-Deficient) by deleting the sucrose and including L-cystine for promoting the growth of cystine dependant dwarf colony coliforms.

### COMPOSITION

Ingredients	Gms / Ltr
Agar	15.000
Lactose	10.000
Peptic digest of animal tissue	4.000
Casein enzyme hydrolysate	4.000
Beef extract	3.000
L-cystine	0.128
Bromo thymol blue	0.020

### PRINCIPLE

Casein enzymatic hydrolysate and Peptic digest of animal tissue acts as a source of amino acids and nitrogen. Beef extract is a source of minerals and vitamins. Lactose is a fermentable sugar and its fermentation results in the release of acid end products that are detected by a pH indicator, bromothymol blue. The indicator changes the colour of medium from green to yellow in acidic conditions, thus differentiating the lactose fermenters from other non-fermenters. L – cystine is added as a growth supplement for cystine dependent coliforms. Agar acts as a solidifying agent. As the electrolytes present in the medium are reduced with omission of sodium chloride, it suppresses the *Proteus* swarming, allowing the vast majority of other coliforms to grow in the medium. Spread plate technique or a dip slide for detection of bacteria in urine is the best technique for CLED Agar.

### INSTRUCTION FOR USE

1. C.L.E.D Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence sterilization is not required.
2. Prior to use, medium in the bottle can be melted either by using a pre-heated water bath or any other method.
3. Slightly loosen the cap before melting.
4. Pour liquefied agar into each plate as desired and allow them to solidify at room temperature. Plates are now ready to inoculate or refrigerate for later use.

### QUALITY CONTROL SPECIFICATIONS



<b>Appearance of the prepared medium</b>	:	Green color, clear to slightly opalescent gel.
<b>Quantity of Medium</b>	:	100 ml of the medium in glass bottle
<b>pH (at 25°C)</b>	:	7.3± 0.2
<b>Sterility Check</b>	:	Passes release criteria

### INTERPRETATION

Cultural characteristics observed after incubation. Recovery rate is considered 100% for bacteria growth on Soya Agar.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Colony appearance	Incubation Temperature	Incubation Period
<i>Enterococcus faecalis</i>	29212	50-100	Luxuriant	>=70%	Slight yellowish or greenish	35-37°C	18-24 Hours
<i>Escherichia coli</i>	25922	50-100	Luxuriant	>=70%	yellow	35-37°C	18-24 Hours
<i>Klebsiella pneumoniae</i>	13883	50-100	Luxuriant	>=70%	Yellow to whitish blue	35-37°C	18-24 Hours
<i>Proteus vulgaris</i>	13315	50-100	Luxuriant	>=70%	Blue	35-37°C	18-24 Hours
<i>Salmonella typhi</i>	6539	50-100	Luxuriant	>=70%	Bluish	35-37°C	18-24 Hours
<i>Staphylococcus aureus</i>	25923	50-100	Luxuriant	>=70%	Deep yellow	35-37°C	18-24 Hours

### PACKAGING

100 ml glass bottles.

### STORAGE

On receipt, store bottles in the dark at 10 to 25° C. Avoid freezing and overheating. The medium may be used up to the expiration date and incubated for the recommended incubation times. Bottles from unopened packages can be used up to the expiration date. Opened bottles must be used immediately. To prepare plates or tubes from the bottled medium, it must first be liquefied. Do not liquefy any leftovers for a second time

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

### DISPOSAL

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

### REFERENCES

1. Brewer J. H., 1942, Science, 95:587.
2. Vera J., 1942, J. Bacteriol., 44:497.
3. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
4. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis, Mo.
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.



**QTY.**  
Quantity

**LOT/  
B. NO.**  
Lot / Batch Number

  
Temperature Unit

  
Manufacturer

  
Best Before

**GMP**  
Certification of  
Good Manufacturing Practices

**REF**  
Catalogue No.

**EC REP** MedNet GmbH  
Barkstrasse 10,  
49163 Moenster, Germany  
Authorized Representative

**CE**  
European Conformity



  
Consults Instructions for use :

**IVD**  
For In Vitro Diagnostic Use

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

**\*For Lab Use Only**  
**Revision: 31<sup>st</sup> March. 2022**

