

CM 21194 - MILK AGAR W/CETRIMIDE (DOUBLE PACK)

INTENDED USE

For cultivation and enumeration of *Pseudomonas aeruginosa* in water.

PRODUCT SUMMARY AND EXPLANATION

Milk Agar was modified by Brown and Scott for the confirmation of *Pseudomonas aeruginosa* in swimming pool waters. Swimming pool water is generally chlorinated potable water but it can also be from thermal springs or salt water. Microorganisms of concern are typically those from the body of the bathers, including the orifices. As *P. aeruginosa* can survive for longer time in water compared to other microorganisms, it is one of the major indicator organisms in the swimming pool. This organism is mainly responsible for ear and eye infection and is very likely to get disseminated in the swimming pool water due to constant contact of ears and eyes with the water. Modified *Pseudomonas* Selective Agar w/Cetrimide is formulated in accordance with ISO Committee under the specifications ISO 8360-1:1988 for the detection and enumeration of *P. aeruginosa* from water. Strains of *P. aeruginosa* are identified by their pigment production i.e. pyocyanin. *P. aeruginosa* is the only species of *Pseudomonas* or gram-negative rod known to excrete pyocyanin.

COMPOSITION

Ingredients	Gms / Ltr
Part I	
SM powder	133.330
Part II	
Peptone	3.330
Sodium chloride	1.670
Yeast extract	1.000
Cetrimide	0.400
Agar	20.000

PRINCIPLE

P. aeruginosa hydrolyzes casein and produces a yellowish to green diffusible pigment on Modified *Pseudomonas* Selective Agar w/ Cetrimide. For isolation, filter 200ml or less water of the swimming pool through sterile membrane filters. Place each membrane filter on M-PA Agar. Incubate the plates at $41.5 \pm 0.5^\circ\text{C}$ for 72 hours. Typical *P. aeruginosa* colonies are 0.8-2.2 mm in diameter, flat in appearance with brownish to greenish centers. For confirmation, using make a single streak from an isolated colony on a Modified *Pseudomonas* Selective Agar w/ Cetrimide plate and incubate at $35-37^\circ\text{C}$ for 24 hours. After incubation *P. aeruginosa* forms pigmented colonies. SM powder, peptone and yeast extract provide all the necessary nutrients mainly nitrogenous for the multiplication of *P. aeruginosa*. *P. aeruginosa* forms yellowish green colonies on this medium. Cetrimide acts as a quaternary ammonium, cationic detergent that causes release of nitrogen and phosphorus from bacterial cells other than *P. aeruginosa*.

INSTRUCTION FOR USE

Dissolve 26.4 grams of Part II in 250 ml purified/distilled water.

Heat to boiling to dissolve the medium completely.

Sterilize by autoclaving at 15 psi pressure (121°C) for 20 minutes. Dissolve 133.33 grams of Part I in 750 ml of purified/distilled water and sterilize by autoclaving at 15 psi pressure (121°C) for 5 minutes.

Cool to $45-50^\circ\text{C}$.

Mix Part I and II and pour into sterile Petri plates.



QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Part I: Off white to cream homogeneous free flowing powder. Part II: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium pH (at 25°C)	: Light amber coloured opalescent gel forms in Petri plates. : 7.3±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Pigment Production	Incubation Temperature	Incubation Period
Escherichia coli	25922	≥103	Inhibited	0%	-	35-37°C	24-48 Hours
Pseudomonas aeruginosa	27853	50-100	Good-luxuriant	≥50%	Blue green	35-37°C	24-48 Hours
Stenotrophomonas maltophilia	13637	≥103	Inhibited	0%	-	35-37°C	24-48 Hours

PACKAGING:

In pack size of 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°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

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
2. Brown M.R.W. and Scott F. J.H., 1970, J. Clin. Pathol., 23:172.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.

