Calcium M- Protein Agar

M1309

Intended use

Recommended for the detection and enumeration of proteolytic microorganisms in foodstuffs and other materials.

Composition**

Ingredients	Gms / Litre
Peptone	4.000
HM extract #	2.000
Tryptone	2.000
Calcinate protein ##	3.500
Calcium chloride, 2H2O	0.200
Tri-potassium citrate, H20	0.350
Disodium hydrogen phosphate	0.105
Potassium dihydrogen phosphate	0.035
Sodium chloride	5.000
Agar	13.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Equivalent to Meat extract

Equivalent to Calcium caseinate

Directions

Suspend 30.19 grams (the equivalent weight of dehydrated medium per litre) in 1000 ml purified/distilled water. Heat gently to boiling with frequent shaking for 10 minutes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix thoroughly while pouring into sterile Petri plates to suspend the precipitate. If desired, to increase turbidity, add 5-10 grams of skim milk powder before heating.

Principle And Interpretation

Protein hydrolysis by microorganisms in foods may produce a variety of odour and flavour defects. On the other hand, microbial proteolytic activity may be desirable in certain foods such as in the ripening of cheese, where it contributes to the development of flavour, body and texture. In some foods the level of proteolytic microorganisms may be of value to predict refrigerated storage life and to assess processing methods (2, 6). Calcium M-Protein Agar is a modification of the original formulation of Frazier and Rupp (3) and is used for the detection and enumeration of proteolytic microorganisms in foodstuffs and other materials.

Tryptone, Peptone and HM extract provides nitrogenous, carbonaceous nutrients along with vitamins and amino acids. Phosphates are added to buffer the medium. Sodium chloride maintains the osmotic equilibrium. Calcinate Protein in the medium is degraded by the proteolytic organisms. This results in formation of clear zones around the proteolytic colonies, in the otherwise opaque medium.

Type of specimen

Food and dairy samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,8). The test sample can be directly surface inoculated or the inoculation can be carried out by the pour plate technique. After an incubation for 24-48 hours, proteolytic organisms, if present will form clear zones on the medium. For better visualization of the zones, the plates can be flooded with 5-10% acetic acid. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets

Limitations :

1. Individual

2. Further biochemical testing is required for complete identification.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.3% Agar gel.

Colour and Clarity of prepared medium

Whitish coloured, turbid gel forms in Petri plates

Reaction

Reaction of 3.02 % w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 2 4 -48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Proteolytic activity
Bacillus cereus ATCC 14579	50-100	good-luxuriant	>=70%	positive, clear zone surrounding colonies
Escherichia coli ATCC 25922 (00013*)	50-100	good-luxuriant	>=70%	negative, no clear zone surrounding colonies
Proteus vulgaris ATCC 13315		good-luxuriant	>=70%	negative, no clear zone surrounding colonies
Pseudomonas aeruginosa ATCC 27853 (00025*)	50-100	good-luxuriant	>=70%	positive, clear zone surrounding colonies

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Chai T., Chen C., Rosen A. and Levin R. E., 1968, Appl. Microbiol.,16: 1738.
2. Frazier W. C. and Rupp P., 1928, J. Bacteriol., 16, 57-63
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.
5. Martely F. G., Jayashankar S. R. and Lawrence, R. C., 1970, J. Appl. Bacteriol., 33: 363.
6. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

Revision : 03/2021