

## Calcium Caseinate Agar

Selective medium for the recovery of proteolytic microorganisms in foods

### Practical information

Applications	Categories
Selective enrichment	Proteolytic microorganisms

Industry: Food

### Principles and uses

Calcium Caseinate Agar is used as a selective medium for the recovery of proteolytic microorganisms in foods. The industrial food processes cause sublethal injuries to many microorganisms and, to aid in recovery, nutrient-rich media are used.

This medium contains casein, a raw milk source rich in amino acids and nitrogen which is degraded by the proteolytes to form clearer zones surrounding the colonies in an otherwise turbid medium. Meat peptone and Beef extract provide nitrogen, vitamins, minerals and amino acids essential for growth. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Calcium hydroxide is the inhibitor. Calcium Chloride helps to maintain the pH of the medium. Bacteriological Agar is the solidifying agent.

### Formula in g/L

Bacteriological agar	13,5	Beef extract	3
Calcium chloride	0,05	Meat peptone	5
Sodium chloride	5	Casein (Hammarsten)	2,5
Calcium hidroxide	0,15		

### Preparation

Suspend 29,2 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. Pour into Petri dishes swirling the medium to resuspend the precipitate. If desired to intensify the turbidity of the medium, 5 to 10 g of skim milk can be added in 1 L of the medium.

### Instructions for use

- Inoculation can be made by streaking the surface of the plate or by using the pour plate method.
- Incubate at  $35 \pm 2^\circ\text{C}$  for 48 - 72 hours. Casein is degraded by proteolytic organisms and forms clear zones surrounding the colonies.
- The finished medium is turbid especially if 5 - 10 g/l of powdered milk is added.
- Count the colonies with clearing zones only. - Covering the surface of the plate with 5 - 10% Acetic acid can improve differentiation of colonies.

### Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
Forms precipitate	Fine powder	Beige	Whitish	$7,2 \pm 0,2$

### Microbiological test

Microrganisms	Specification
Bacillus cereus ATCC 11778	Good growth with transparency halo
Enterobacter cloacae ATCC 13047	Good growth without transparency halo

Proteus vulgaris ATCC 13315  
Escherichia coli ATCC 25922  
Pseudomonas aeruginosa ATCC 27853

Good growth without transparency halo  
Good growth without transparency halo  
Good growth with transparency halo

## Storage

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Temp. Min.:2 °C  
Temp. Max.:25 °C

## Bibliography

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Frazier, W.C., a. RUPP, P: Studies on the proteolytic bacteria of milk. A. medium for the direct isolation of caseolytic milk bacteria. J. Bact. 16 57-63 (1928).

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