

# Calcium Caseinate Agar acc. to FRAZIER and RUPP, modified

A modification of the selective agar proposed by FRAZIER and RUPP (1928) for the detection and enumeration of proteolytic microorganisms (proteolytes) in foodstuffs and other materials.

## Mode of Action

This medium contains casein which is degraded by the proteolytes to form clearer zones surrounding the colonies in the otherwise turbid medium.

## Typical Composition (g/litre)

Peptone from meat 4.0; meat extract 2.0; peptone from casein 2.0; calcium caseinate 3.5; calcium chloride dihydrate 0.2; tri-potassium citrate monohydrate 0.35; di-sodium hydrogen phosphate anhydrous 0.105; potassium dihydrogen phosphate 0.035; sodium chloride 5.0; agar-agar 13.0.

## Preparation

Suspend 30.2 g/litre completely (if necessary use a mixer), place in a cold water bath and while frequently shaking heat slowly until the suspension boils, boil for about 10 minutes, autoclave (15 min at 121 °C). Mix thoroughly while pouring to suspend the precipitate. 5-10 g skim milk powder/litre can be added before heating to increase turbidity.

pH: 7.0 ± 0.2 at 25 °C.

The plates are turbid and yellowish-brown.

## Experimental Procedure and Evaluation

Inoculate by the pour-plate method or by spreading the sample on the surface of the medium.

Incubation: 2-3 days at 35 °C aerobically.

Count the proteolyte colonies (surrounded by clear zones). The plates can be flooded with 5 to 10 % acetic acid to facilitate recognition of the zones.

## Literature

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

## Ordering Information

Product	Merck Cat. No.	Pack size
Calcium Caseinate Agar acc. to FRAZIER and RUPP, modified	1.05409.0500	500 g
Acetic acid min. 96 %	1.00062.1000	1 l
Skim milk powder	1.15363.0500	500 g

## Quality control

Test strains	Growth	Clear zone
<i>Bacillus cereus</i> ATCC 11778	good / very good	+
<i>Pseudomonas aeruginosa</i> ATCC 27853	good / very good	+
<i>Proteus vulgaris</i> ATCC 13315	good / very good	-
<i>Escherichia coli</i> ATCC 25922	good / very good	-
<i>Enterobacter cloacae</i> ATCC 13047	good / very good	-



*Enterobacter cloacae*  
ATCC 13047



*Proteus vulgaris*  
ATCC 13315