91365 Reinforced Clostridial Agar

Used for the cultivation and enumeration of Clostridia.

Composition:

Ingredients	Grams/Litre
Casein enzymatic hydrolysate	10.0
Beef extract	10.0
Yeast extract	3.0
Dextrose	5.0
Sodium chloride	5.0
Sodium acetate	3.0
Starch soluble	1.0
L-Cysteine hydrochloride	0.5
Agar	13.5
Final pH 6.8 +/- 0.2 at 25°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Suspend 51 g of Reinforced Clostridial Agar in 1000 ml of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 10 lbs. Pressure (115°C) for 15 minutes.

Principle and Interpretation:
Reinforced Clostridial Agar is based on the original formulation from Hirsch and Grinsted. It can be used tio initiate growth from small inocula and to obtain the highest viable count of Clostridia. This medium can be used like the conventional medium for studies of spore forming anaerobes, especially *Clostridium butyricum* in chesse and general for the enumeration and isolation of Clostridia. Other spore forming anaerobes like streptococci and lactobacilli grow as well on this media.
Casein hydrolysate, beef extract, yeast extract and peptone provide nitrogen, vitamins, amino acids and carbon for growth. Dextrose is the fermentable sugar and sodium chloride ensures osmotic balance. The medium is free from inhibitors and contains cysteine as a reducing agent. Sodium acetate is the buffering agent. Polymyxin B can be added to inhibit Gram-negative bacteria and to make the medium more selective [1].

Cultural characteristics after incubation at 35-37°C for 40-48 hours, in an anaerobic atmosphere.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery
Bacteroides fragilis (23745)	10^2-10^3	+++	> 70%
Bacteroides vulgatus (8482)	10^2-10^3	+++	> 70%
Clostridium butyricum (9690)	10^2-10^3	+++	> 70%
Clostridium perfringens (13124)	10^2-10^3	+++	> 70%

References:
1. A. Hirsch, E. Grinsted, Methods for the growth and enumeration of anaerobic spore formers from cheese, with observations on the effect of nisin. J. Dairy Res., 21, 101-110 (1954)
2. Ella M. Barnes, M. Ingram, J. Appl. Bact., 19, 117-128 (1956)
3. K.H. Lewis, R. Angelotti, Examination of Foods for Enteropathogenic and Indicator Bacteria, US Dept. of Health, Education and Welfare, Washington, DC (1964)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.