



Casein Hydrolysate Broth

M200

Casein Hydrolysate Broth is used for production of Staphylococcal enterotoxin for use in cat test and in serological studies.

Composition**

Ingredients	Gms / Litre
Casein acid hydrolysate	20.000
Ferric citrate	0.025
Potassium phosphate	2.000
Magnesium sulphate	0.200
L-Cystine	0.025
Sodium acetate	7.000
L-Tryptophan	0.075
Calcium pantothenate	0.0005
Thiamine	0.00004
Nicotinic acid	0.0012
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 29.33 grams in 1000 ml distilled water. Mix thoroughly. Heat with frequent agitation and boil for one minute. Dispense as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Casein Hydrolysate Broth was developed by Casman (1) and is used for production of Staphylococcal enterotoxin for use in the cat test and in serological studies.

As described in APHA (2), Staphylococci to be tested for enterotoxigenicity should be subcultured into the tubes of Casein Hydrolysate Broth (2) and incubated in an atmosphere containing 30% Carbon - dioxide for 18 - 24 hours at 35°C. Growth obtained by this method is then transferred from each tube in three ml amounts to duplicate flasks containing 100 ml Casein Hydrolysate Broth and the flasks should be incubated as mentioned above for three days. The broth cultures are then centrifuged and supernatant fluid is sterilized by Seitz filtration. The filtrates are then tested for alpha and beta haemolysins and if present then the toxins are denatured by heat or by neutralization with antiserum. After denaturation, filtrates can be injected in the cats to observe if vomiting is induced. Casein Hydrolysate Broth can be solidified with the addition of agar and the cultures grown are used for tests on other animals and in analysis of antigen-antibody systems by agar diffusion technique (3).

Quality Control

Appearance

Cream to yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium

Light to medium amber coloured clear to slightly opalescent solution with slight precipitate

Reaction

Reaction of 2.93% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Cultural Response

Organism	Inoculum (CFU)	Growth
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Cultural Response

<i>Enterococcus faecalis</i> ATCC 50-100 29212	luxuriant
<i>Staphylococcus aureus</i> 50-100 ATCC 25923	luxuriant

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

- 1.Casman, 1958, Public Health Reports, 73:599.
- 2.Standard Methods for the Examination of Dairy Products, 1960, 11th ed., American Public Health Association, Inc. New York, 1960.
- 3.Casman, 1960, J. Bact., 79:849.

Revision : 1 / 2011

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