

**CASEIN HYDROLYSATE AGAR W/ 2.5% AGAR****TM 692**

For large scale cultivation of *Vibrio cholerae* for cholera vaccine production

Composition

Ingredients	Gms/Ltr.
Beef, infusion from	150.00
Agar	25.00
Casein enzymatic hydrolysate	5.00
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Sodium phosphate	2.50
Yeast autolysate	1.50

* Dehydrated powder, store in a dry place, in tightly-sealed containers at 24°C and protect from direct sunlight.

[1.5gm of Beef is equivalent to 150gm of Beef, infusion from]**

Instructions for Use

Dissolve 45.50gms in 1000ml of distilled water which already contains 22ml of glycerol. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to 45 - 50°C mix well before pouring into the Petri plates.

Appearance: Light yellow colour, slightly opalescent gel

pH (at 25°C): 7.8 ± 0.2

Principle

CASEIN HYDROLYSATE AGAR W/ 2.5% AGAR is used for large scale cultivation of *Vibrio cholerae* for cholera vaccine production. The medium compositions are according to the recommendations and regulations of APHA. *Vibrio cholerae* is Gram negative, facultatively anaerobic pathogenic bacteria and the causative agent of the disease cholera. The organism colonizes in the small intestine and secretes a protein, cholera toxin that causes profuse, watery diarrhea. This medium contains Peptic digest of animal tissue, Casein enzymatic hydrolysate and Beef infusion from to provide the carbonaceous and nitrogenous compounds to support the growth of microbes in the medium. Yeast autolysate can provide the essential vitamins and other necessary growth factors for the organisms. Glycerol is used as source of carbohydrate and energy required for the growth of the microorganisms in the medium. The Sodium chloride can control the osmotic balance of the medium while the Sodium phosphate can act as a buffering agent to maintain the pH of the medium favourable for the growth of the microorganisms. Agar is added as a solidifying agent of the medium.

Interpretation

Cultural characteristics observed after inoculating the organisms and subsequent incubation at 35 – 37°C for 18 – 48 hours.

PRODUCT DATA SHEET

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
<i>Vibrio cholerae</i>	15748	$10^3 - 10^5$	Luxuriant
<i>Escherichia coli</i>	25922	$10^3 - 10^5$	Luxuriant
<i>Salmonella typhi</i>	6539	$10^3 - 10^5$	Luxuriant

References

1. Vanderzant C and Splittstoerser D (Eds). 1992. Compendium of Methods for the Microbiological Examination of Foods, 3rd ed, APHA, Washington, DC.