PRODUCT SPECIFICATION SHEET

Anaerobic Egg Agar Base (DM497)

Intended Use
Anaerobic Egg Agar Base (DM497) is recommended for detection of *Clostridium perfringens* in food samples, in compliance with FDA BAM, 1998.

Product Summary and Explanation
Clostridium species are anaerobic, spore forming, gram-positive rods occurring naturally in soil.[1] *Clostridium perfringens*, ranked behind *Salmonella* species and *Staphylococcus aureus*, has been the third most common etiological agent of food-borne disease.[2] *C. perfringens* food poisoning results from eating contaminated food. *C. perfringens* infections often occur when foods are prepared in large quantities and are then kept warm for a long time before serving. The major virulence factor of *C. perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses.[1] If the suspected food samples are refrigerated, *C. perfringens* cells may lose viability, thereby making it difficult to convict the organisms in food poisoning outbreaks.[3] Anaerobic Egg Agar is one of the media recommended by APHA[4] for detecting *C. perfringens* in foods.

Principles of the Procedure
Anaerobic Egg Agar Base contains casein enzymic hydrolysate and proteose peptone which provides amino acids and other complex nitrogenous nutrients. Yeast extract provides essential B-complex vitamins. Egg yolk emulsion is added to the medium by which the lipase and lecithinase activity can be observed. Sodium chloride maintains the osmotic balance of the medium.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	20.00
Casein enzymic hydrolysate	5.00
Yeast extract	5.00
Sodium chloride	5.00
Agar	20.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 55 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Cool to 45-50°C and aseptically add 80 ml sterile Egg Yolk Emulsion (MS038).
5. Mix thoroughly before pouring into sterile Petri plates.

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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium : Light yellow coloured, clear to very slightly opalescent gel After addition of Egg Yolk Emulsion : Light yellow coloured, opaque gel forms in Petri plates
Reaction of 5.5% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.0% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Egg Yolk Emulsion (MS038) when incubated anaerobically,at 35-37°C for 18-24 hours.

Sr. No.	Organisms	Results to be achieved				
		Inoculum (CFU)	Growth	Recovery	Lecithinase	Lipase
1.	*Clostridium perfringens ATCC 12924*	50 -100	good-luxuriant	>=50%	positive reaction, opaque zone around the colony	negative reaction
2.	*Clostridium sporogenes ATCC 11437*	50 -100	good-luxuriant	>=50%	negative reaction	positive reaction, iridescent sheen on the colony

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure
Refer to appropriate references for standard test procedures.

Results
1. Lecithinase activity is observed as formation of an insoluble opaque precipitate, which results when lecithinase of *C. perfringens* degrades lecithin of egg yolk.
2. Lipase reaction is observed as iridescent sheen formed on colonies due to breakdown of free fats present in the egg yolk by lipase. For the lipase reaction, plates may be kept upto a week for incubation.
3. Proteolysis is indicated by clear zones in the medium surrounding the growth.

Storage
Store the sealed bottle containing the dehydrated medium at 10 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration
Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure
1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

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Packaging
Product Name : Anaerobic Egg Agar Base
Product Code : DM497
Available Pack sizes : 500gm

References

1. Czeczulin J. R., Hanna P. C., Mcclane B., 1993, Infect. Immun., 61: 3429-3439.
2. Centre for Disease Control, 1982, CDC Surveillance Summaries, 35:7SS-16SS, 1986.
3. Traci P. A., and Duncan C. L.,1974 , Appl. Microbiol., 28:815
4. Downes F. P. and Ito K., (Eds.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th Ed., APHA, Washington, D.C.

Further Information

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