PRODUCT SPECIFICATION SHEET

Egg Yolk Agar Base (DM341)

Intended Use
Egg Yolk Agar Base (DM341) is recommended for isolation and identification of *Clostridia* and certain other anaerobes.

Product Summary and Explanation
Food poisoning caused by *Clostridium perfringens* is one of the most common types of human food borne illness.[1] The foods usually involved are cooked meat or poultry products containing large numbers of viable cells. In perfringens poisoning, a heat-labile enterotoxin produced only by sporulating cells[2] induces the major symptoms of diarrhea. Egg Yolk Agar, Modified is based on an egg yolk medium developed by McClung and Toabe for the isolation and presumptive differentiation of clostridia based on lecithinase and lipase production and proteolytic activity. Egg Yolk Agar Base differs from the original formula by the inclusion of hemin.[3,4]

Principles of the Procedure
Egg Yolk Agar, Modified contains proteose peptone which provides essential nutrients along with carbonaceous and nitrogenous substances. Disodium phosphate and Monopotassium phosphate buffers the medium.Sodium chloride maintains the osmotic equilibrium. Magnesium sulphate serves as a source of divalent cations along with sulphates. Glucose serves as a source of energy. Hemin helps to improve the growth of anaerobic organisms. An egg yolk suspension is incorporated to detect the production of lecithinase and lipase and proteolytic activity.

Formula / Liter

Ingredients	Gms / Liter
Proteose peptone	40.00
Disodium phosphate	5.00
Monopotassium phosphate	1.00
Sodium chloride	2.00
Magnesium sulphate	0.10
Glucose	2.00
Hemin	0.005
Agar	25.00
Final pH: 7.6 ± 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 75.10 grams of the medium in 900 ml of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense in 90 ml amounts and sterilize by autoclaving at 121°C 15 lbs pressure, for 15 minutes / validate cycle.
4. Cool to 45-50°C and add 10 ml of sterile egg yolk emulsion (MS038) per 90 ml of medium.
5. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium: Medium amber coloured, clear to slightly opalescent gel After addition of egg yolk emulsion (MS038): Yellow coloured opaque gel forms in Petri plates
Reaction of 7.5% solution	pH 7.6 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 2.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added Egg yolk emulsion (MS038), after an incubation at 35-37°C for 48-72 hours when incubated anaerobically. (*- Plates should be incubated up to 7 days before regarding them as negative)

Sr. No.	Organisms	Results to be achieved					
		Inoculum (CFU)	Growth	Recovery	Lecithinase	*Lipase Activity	Proteolytic Activity
1.	*Bacteroides fragilis ATCC25285*	50-100	good-luxuriant	>=50%	negative reaction	negative, no irridescent sheen on the colony surface and medium	negative, no clear zone surrounding colonies
2.	*Clostridium botulinum ATCC 25763*	50-100	good-luxuriant	>=50%	negative reaction	negative, no irridescent sheen on the colony surface and medium	positive, clear zone surrounding colonies
3.	*Clostridium butyricum ATCC 13732*	50-100	good-luxuriant	>=50%	negative reaction	negative, no irridescent sheen on the colony surface and medium	positive, clear zone surrounding colonies
4.	*Clostridium perfringens ATCC 12924*	50-100	good-luxuriant	>=50%	positive, opaque zone around the colony	negative, no irridescent sheen on the colony surface and medium	negative,no clear zone surrounding colonies
5.	*Clostridium sporogenes ATCC 11437*	50-100	good-luxuriant	>=50%	negative reaction	positive, irridescent sheen on the colony surface and medium	positive, clear zone surrounding colonies

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

Test Procedure

1. The test specimen should be directly inoculated on the media.
2. Prior to inoculation, media plates should be reduced by placing in an anaerobic jar for 18-24 hours.
3. Simultaneously inoculate an enrichment broth with the test sample to detect small number of anaerobic organisms.
4. Standard procedures for the isolation of organism should be referred.
5. Incubation should be carried out for 18-48 hours and continued for 7 days.

Results

1. Organisms producing lecithinase break down lecithin present in the egg yolk emulsion producing an insoluble opaque precipitate around the colonies.
2. Lipase-producing organisms break down free fatty acids (in the egg yolk emulsion) forming an iridescent (oil on water) sheen on the surface of the colonies.
3. Lipase activity may be delayed, therefore plates should not be discarded as negative before incubation for a week.
4. Proteolytic activity is seen as clear zones around the colonies.

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.

Packaging
Product Name : Egg Yolk Agar Base
Product Code : DM341
Available Pack sizes : 100gm / 500gm

References
1. Labbe R., 1989, Clostridium perfringens, In Foodborne Bacterial Pathogens Ed., Doyle M. P., P.191, Marcel Dekker, New York , N.Y.,
2. Duncan C. L., 1973, A. J. Bacteriol., 113:932
3. Atlas R. M., 2004, Handbook of Microbiological Media, 3rd Ed., CRC Press.
4. McClung and Toabe, 1947, J. Bacteriol., 53:139

Further Information
For further information please contact your local MICROMASTER Representative.

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