PRODUCT SPECIFICATION SHEET

Phenylalanine Agar (DM210)

Intended Use

Phenylalanine Agar (DM210) is used for the differentiation of Proteus and Providencia group of organisms from other members of Enterobacteriaceae on the basis of their ability to form Phenyl Pyruvic acid from phenylalanine.

Product Summary and Explanation

Henrickson demonstrated during his initial trials that *Proteus* species were able to transform phenylalanine to phenylpyruvic acid.[1] Singer and Volcani,[2] Hamida and LeMinor[3] and other researchers studied the reaction and highlighted its usefulness in the taxonomy of the *Enterobacteriaceae*. A culture medium containing phenylalanine was developed by Buttiaux et al, to study the characteristic biochemical properties of the *Proteus* and *Providencia* genera.[4] This medium was designed to differentiate members of the *Proteeae* from other members of the *Enterobacteriaceae* by the ability of organisms in the genera within the *Proteeae* to deaminate phenylalanine to phenylpyruvic acid by enzymatic activity[5]*Proteus, Providencia* and *Morganella* species possess this capability. This formula conforms to the modified formula of Ewing et al. [6] Ferric Chloride Reagent is used to determine if a specific microorganism is capable of producing phenylpyruvic acid from phenylalanine. [5]

Principles of the Procedure

The growth of the organisms in the medium is supported by Yeast extract which serves as the main source of nitrogen and vitamins. Sodium chloride maintains osmotic equilibrium. The phenylalanine serves as the substrate for enzymes, which are able to deaminate it to form phenylpyruvic acid. The addition of 3-5 drops of a 10% aqueous ferric chloride solution (or a 12% aqueous ferric chloride solution acidified with 2.5 mL of concentrated HCl per 100 mL of reagent) to the cultures following incubation, results in the appearance of a light to deep green color (positive reaction) or no color change (negative reaction). In a positive reaction, any phenylpyruvic acid present will react with the ferric salt in the reagent to give a green color.

Formula / Liter

Ingredients	Gms / Liter
Yeast extract	3.00
Sodium chloride	5.00
DL-Phenylalanine	2.00
Disodium phosphate	1.00
Agar	15.00
Final pH: 7.3 ± 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 26 grams of the medium in one liter of distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Dispense in tubes and autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Allow the tubed medium to cool in a slanting position.

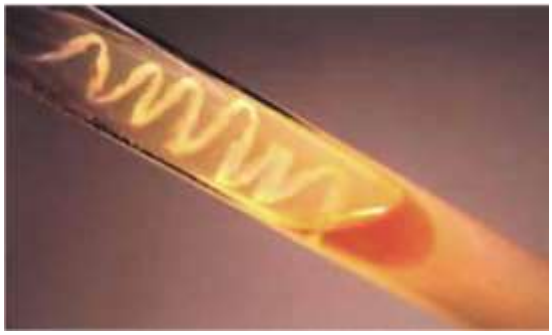
Quality Control Specifications

Dehydrated Appearance	Cream to yellow colored, homogeneous, free flowing powder
Prepared Medium	Light amber coloured slightly opalescent gel forms in tubes as slants
Reaction of 2.6 % Solution	pH : 7.3 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed after an incubation at 35 - 37°C for 12 - 16 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Phenylalanine deaminase
1.	*Enterobacter aerogenes ATCC 13048*	50 -100	Luxuriant	negative reaction
2.	*Escherichia coli ATCC25922*	50 -100	Luxuriant	negative reaction
3.	*Proteus mirabilis ATCC25933*	50-100	Luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
4.	*Proteus vulgaris ATCC13315*	50-100	Luxuriant	positive reaction, green colouration after addition of 10% ferric chloride
5.	*Providencia alcalifaciens ATCC 9886*	50-100	Luxuriant	positive reaction, green colouration after addition of 10% ferric chloride

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



Escherichia coli ATCC 25922 on Phenylalanine Agar (DM210)

Proteus mirabilis ATCC25933 on Phenylalanine Agar (DM210)

Test Procedure

1. Inoculate tubed slants, using a heavy inoculum, with growth from an 18 to 24-hour pure culture.
2. Incubate tubes aerobically at 35 ± 2°C for 4 hours or 18-24 hours.
3. If the inoculum is sufficiently heavy, a 4-hour incubation period should be adequate.[5]

Results

1. After the incubation period, add 3-5 drops of the ferric chloride reagent to the slants.
2. Gently rotate the tube to loosen the growth.
3. Observe for the production of a green color (positive reaction) within 1-5 minutes.
4. Members of *Proteus, Morganella* and *Providencia* genera produce positive results.
5. Most other genera within the *Enterobacteriaceae* are negative for phenylpyruvic acid production.[7,8]

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. A positive reaction must be interpreted within the first 5 minutes after addition of the reagent, as the green color fades rapidly.
2. A few other strains of Enterobacteriaceae are also phenylalanine positive: Enterobacter agglomerans (20%), Enterobacter sakazakii (50%), Rahnella aquatilis (95%), and Tatumella ptyseos (90%).[9]
3. For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed
4. For final identification. Consult appropriate texts for detailed information and recommended procedures.[7-9]

Packaging

Product Name : Phenylalanine Agar

Product Code : DM210

Available Pack sizes : 100gm / 500gm

References

1. Henrikson. 1950. J. Bacteriol. *60*:225.
2. Singer and Volcani. 1955. J. Bacteriol. *69*:303.
3. Hamida and LeMinor. 1956. Ann. Inst. Pasteur. *90*:671.
4. Buttiaux, Osteux, Fresnoy and Moriamez. 1954. Ann. Inst. Pasteur Lille. *87*:375.
5. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
6. Ewing, Davis and Reavis. 1957. Public Health Lab. *15*:153.
7. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
8. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed American Society for Microbiology, Washington, D.C.
9. Farmer, J.J., III. 1999. Enterobacteriaceae: introduction and identification, p. 442-458. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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