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Decarboxylase Test Medium Base (Falkow) (DM638)

Intended Use
Decarboxylase Test Medium Base (Falkow) (DM638) is recommended for testing amino acid decarboxylase activity of bacteria.

Product Summary and Explanation
Decarboxylase media was first introduced by Moeller for differentiating bacteria on their ability to decarboxylate the amino acids and detecting the production of lysine decarboxylase, ornithine decarboxylase and arginine dihydrolase.[1-3] These media are a useful adjunct to other biochemical tests for the speciation and identification of the *Enterobacteriaceae* and other gram-negative bacilli.[4-8] Moellers work was based on the experiments done by Gale[9] and Gale and Epps[10] on bacterial amino acid decarboxylases. Moeller observed that production of lysine, arginine, ornithine decarboxylase by various members of *Enterobacteriaceae* offered an important parameter to other biochemical tests for differentiating bacteria within closely related groups. A medium utilizing the lysine decarboxylase reaction was further developed to differentiate *Salmonella* serotype Arizonae from *Citrobacter*, Calquist.[11] Falkow[12] later accentuated and developed the lysine decarboxylase medium for differentiating *Salmonellae* and *Shigellae* by the valid and reliable results.

Principles of the Procedure
Decarboxylase Test Medium Base (Falkow) contains peptic digest of animal tissue and yeast extract which supply the nitrogenous, vitamins and other nutrients necessary to support bacterial growth. Dextrose is a fermentable carbohydrate. Bromcresol purple and cresol red are pH indicators. The amino acids lysine, ornithine or arginine are added to the basal medium to detect the production of the enzyme specific for these substrates. When the medium is inoculated with a bacterium that is able to ferment dextrose, acids are produced that lower the pH of the medium and change the colour of the indicator from purple to yellow. The acidic condition also stimulates decarboxylase activity. If the organism produces the appropriate enzyme, the amino acid in the medium is degraded, yielding a corresponding amine. Decarboxylation of lysine yields cadaverine, while decarboxylation of ornithine yields putrescine. Arginine is first hydrolyzed to form ornithine, which is then decarboxylated to form putrescine. The production of these amines elevates the pH of the medium, changing the colour of the indicator from yellow to purple or violet.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	5.00
Yeast extract	3.00
Dextrose	1.00
Bromocresol purple	0.02
Final pH : 6.7 ± 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 9.02 grams of the medium in one liter of distilled water.
2. Heat, if necessary to dissolve the medium completely.
3. Divide into four equal parts. One part is tubed without addition of any amino acid. To the remaining three parts, add separately 3 amino acids, L-lysine hydrochloride, L-arginine hydrochloride and L-ornithine hydrochloride to a final concentration of 0.5%.
4. Dispense in 3-4 ml quantities in screw capped tubes. Autoclave at 121°C, 15 psi pressure, for 15 minutes.

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5. To avoid false alkalinization at the surface of medium it is recommended to add liquid paraffin to a height of about 5mm before sterilization.

Quality Control Specifications

Dehydrated Appearance	Light yellow to greenish yellow homogeneous free flowing powder
Prepared Medium	Purple coloured, clear solution without any precipitate in tubes
Reaction of 0.9% Solution	pH : 6.7 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for upto 4 days with addition of appropriate amino acids and overlaying with sterile mineral oil.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Arginine decarboxylation	Ornithine decarboxylation	Lysine decarboxylation
1.	*Citrobacter freundii ATCC 8090*	50 -100	variable reaction	variable reaction	negative reaction, yellow colour
2.	*Enterobacter aerogenes ATCC 13048*	50 -100	negative reaction, yellow colour	positive reaction, purple colour	positive reaction, purple colour
3.	*Escherichia coli ATCC 25922*	50 -100	variable reaction	variable reaction	positive reaction, purple colour
4.	*Klebsiella pneumoniae ATCC 13883*	50 -100	negative reaction, yellow colour	negative reaction, yellow colour	positive reaction, purple colour
5.	*Proteus mirabilis ATCC 25933*	50 -100	negative reaction, yellow colour	positive reaction, purple colour	negative reaction, yellow colour
6.	*Proteus vulgaris ATCC 13315*	50 -100	negative reaction, yellow colour	negative reaction, yellow colour	negative reaction, yellow colour
7.	*Salmonella Paratyphi A ATCC 9150*	50 -100	delayed positive reaction / positive reaction, purple colour	positive reaction, purple colour	negative reaction, yellow colour
8.	*Salmonella Typhi ATCC 6539*	50 -100	delayed positive reaction / negative reaction	negative reaction, yellow colour	positive reaction, purple colour
9.	*Serratia marcescens ATCC 8100*	50 -100	negative reaction, yellow colour	positive reaction, purple colour	positive reaction, purple colour
10.	*Shigella dysenteriae ATCC 13313*	50 -100	negative reaction/ delayed positive reaction	negative reaction, yellow colour	negative reaction, yellow colour
11.	*Shigella flexneri ATCC 12022*	50 -100	negative reaction/ delayed positive reaction	negative reaction, yellow colour	negative reaction, yellow colour
12.	*Shigella sonnei ATCC 25931*	50 -100	variable reaction	positive reaction, purple colour	negative reaction, yellow colour

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

Test Procedure

1. Inoculate the broth media by transferring one or two colonies from the surface of a fresh culture with an inoculating loop or needle and mix to distribute the culture throughout the medium.
2. Inoculated tubes must be protected from air (by overlaying the medium with 1 ml sterile mineral oil) to avoid false alkalinization at the surface of the medium. Control tubes of basal media should be inoculated.

3. Incubate the tubes with caps tightened at 35 ± 2°C. Examine for growth and decarboxylase reactions after 18-24, 48, 72 and 96 hours before reporting as negative.
4. The medium will become yellow initially, if the dextrose is fermented, and then will gradually turn purple if the decarboxylase or dihydrolase reaction occurs and elevates the pH turning the medium alkaline

Results

1. Compare the color of tubes of media containing the specific amino acids with the color of control tubes of basal media (without amino acid) that have been inoculated with the same isolate.
2. If inoculated control tubes show an alkaline reaction, the test is invalid; i.e., either improperly performed or the test organisms can degrade the peptone sufficiently to produce an alkaline reaction in the absence of a specific amino acid.
3. The medium becomes purple to violet if the reaction is positive (alkaline).
4. A yellow color indicates a negative test; i.e., the organism does not produce the appropriate enzyme.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 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. Biochemical testing should be attempted on pure culture isolation only and subsequent to differential determinations.
2. The decarboxylase reactions can be considered indicative of a given genus or species but conclusive and final identification of these organisms cannot be made solely on the basis of the decarboxylase reactions.
3. If layers of yellow and purple appear after incubation, shake the test tube gently before attempting to interpret results.
4. If a reaction is difficult to interpret, compare the tube in question to an uninoculated control tube. Any trace of purple after 24 hours of incubation is a positive test.
5. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
6. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Decarboxylase Test Medium Base (Falkow)
Product Code : DM638
Available Pack sizes : 100gm / 500gm

References

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Further Information

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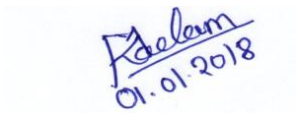	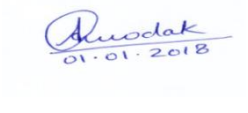	
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