PRODUCT SPECIFICATION SHEET

Decarboxylase Broth Base, Moeller (Moeller Decarboxylase Broth Base) (DM132)

Intended Use
Decarboxylase Broth Base, Moeller (Moeller Decarboxylase Broth Base) (DM132) with the addition of appropriate L-amino acid, it is used to differentiate bacteria on the basis of their ability to decarboxylate the amino acid.

Product Summary and Explanation
Moeller Decarboxylase Broth Base is used for differentiating gram-negative enteric bacilli on the basis of their ability to decarboxylate amino acids. Decarboxylase tests are important in the differentiation and identification of a wide variety of microorganisms and are outlined in numerous standard methods.[1-4] Decarboxylase media was introduced by Moeller for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase.[5-8] These media are a useful adjunct to other biochemical tests for the speciation and identification of the *Enterobacteriaceae* and other gram-negative bacilli.[8-12] Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale[13] and Gale and Epps.[14] The production of ornithine decarboxylase is particularly useful for differentiating *Klebsiella* and *Enterobacter* species. *Klebsiella* species are non-motile and, except for *K. ornithinolytica*, do not produce ornithine decarboxylase, while most *Enterobacter* species are motile and, except for *E. agglomerans*, usually produce this enzyme.[10] Falkow obtained valid and reliable results with a lysine decarboxylase medium he developed to differentiate and identify *Salmonella* and *Shigella*.[15] Although his modification of the Moeller formula was originally described as a lysine medium only, further study by Falkow and then by Ewing, Davis and Edwards,[16] substantiated the use of the medium for ornithine and arginine decarboxylase reactions as well. Ewing, Davis and Edwards[16] compared the Falkow decarboxylase medium base to the Moeller medium and reported that, although the two methods compared favorably in most cases, the Moeller medium was found to be more reliable for cultures of *Klebsiella* and *Enterobacter*. They concluded that the Moeller method should be regarded as the standard or reference method, although the Falkow formula is suitable for determining decarboxylase reactions for most members of the *Enterobacteriaceae* except for *Klebsiella* and *Enterobacter*. The Moeller medium is also particularly useful in the identification of *Aeromonas*, *Plesiomonas*, *Vibrio* spp. and nonfermentative gram-negative bacilli.[17] Decarboxylase Base Moeller conforms with the Moeller formulation while Decarboxylase Medium Base is prepared according to the formula described by Falkow.

Principles of the Procedure
Decarboxylase Broth Base, Moeller contains beef extract and peptic digest of animal tissue, which provide nitrogenous and other nutrients necessary to support bacterial growth. Dextrose is the fermentable carbohydrate and pyridoxal is the co-factor for the decarboxylase enzyme. Bromo cresol purple and cresol red are the pH indicators in this medium. When the medium is inoculated with the dextrose fermenting bacteria, the pH is lowered due to acid production, which changes the colour of the indicator from purple to yellow. Acid produced in turn stimulates decarboxylase enzyme. Decarboxylation of lysine yields cadaverine while putrescine is produced due to ornithine decarboxylation. Arginine is first hydrolyzed to ornithine which is then decarboxylated to form putrescine. Formation of these amines increases the pH of the medium, changing the colour of the indicator from yellow to purple. If the organisms do not produce the appropriate enzyme, the medium remains acidic, yellow in colour. Each isolate to be tested must also be inoculated into a tube of the basal medium that does not contain the amino acid. If this tube becomes alkaline, the test is invalid. To obtain the appropriate reactions, the inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalinization at the surface of the medium, which could cause a decarboxylase-negative organism to appear positive.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of soyabean meal	5.00
Beef extract	5.00
Dextrose	0.50

Bromocresol purple	0.01
Cresol red	0.005
Pyridoxal	0.005
Final pH: 6.0 ± 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 10.52 grams of the medium in one liter of distilled water.
2. Add 10 grams of L-Lysine, L-Arginine, L-Ornithine or other L-amino acids. When using DL-amino acids, use 2% concentration.
3. Heat to boiling, to dissolve the medium completely.
4. When L-Ornithine is added, readjustment of the pH may be required.
5. Dispense in 5 ml amount in screw-capped tubes.
6. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

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 1.05% Solution	pH : 6.0 ± 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 pneumonia 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

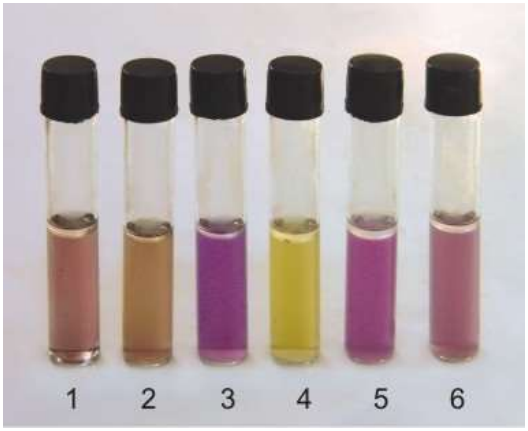
The organisms listed are the minimum that should be used for quality control testing.

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. Overlay the medium in each tube with 1 mL sterile mineral oil.
3. Incubate the tubes with caps tightened at 35 - 37°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.

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. The test is invalid, if inoculated control tubes show an alkaline reaction; 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 decarboxylation reaction is positive (alkaline).
4. A yellow color indicates a negative test; i.e., the organism does not produce the appropriate enzyme.



Moeller Decarboxylase Broth Base (DM132)

Results with added L-Lysine

1. Control
2. Citrobacter freundii ATCC 8090
3. Escherichia coli ATCC 25922
4. Proteus mirabilis ATCC 25933
5. Enterobacter aerogenes ATCC 13048
6. Klebsiella *pneumonia ATCC 13883*

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. If isolated or received on a selective medium, the organism should be subcultured to Casein Soyabean Digest Agar with 5% Sheep Blood or other suitable culture medium before attempting to determine decarboxylase or dihydrolase activity.

2. Biochemical characteristics of the *Enterobacteriaceae* serve to confirm presumptive identification based on cultural, morphological, and/or serological findings. Therefore, biochemical testing should be attempted on pure culture isolates only and subsequent to differential determinations.
3. The decarboxylase reactions are part of a total biochemical profile for members of the *Enterobacteriaceae* and related organisms. Results obtained from these reactions, therefore, can be considered presumptively indicative of a given genus or species. However, conclusive and final identification of these organisms cannot be made solely on the basis of the decarboxylase reactions.
4. If layers of yellow and purple appear after incubation, shake the test tube gently before attempting to interpret results.
5. Compare the tube in question to an uninoculated control tube, if a reaction is difficult to interpret. Any trace of purple after 24 hours of incubation is a positive test.
6. A gray color may indicate reduction of the indicator. Additional indicator may be added before the results are interpreted.
7. *Salmonella gallinarum* gives a delayed positive ornithine decarboxylase reaction, requiring 5-6 days incubation. Many strains of *E. coli*, including those that ferment adonitol, may exhibit a delayed reaction.

Packaging
Product Name : Decarboxylase Broth Base, Moeller (Moeller Decarboxylase Broth Base)
Product Code : DM132
Available Pack sizes : 100gm/ 500gm

References

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Further Information

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