Decarboxylase Differential Media
Decarboxylase Base Moeller • Decarboxylase Medium Base • Lysine Decarboxylase Broth • Moeller Decarboxylase Broth Base • Moeller Decarboxylase Broth with Arginine • Moeller Decarboxylase Broth with Lysine • Moeller Decarboxylase Broth with Ornithine

Intended Use

Decarboxylase media are used in the biochemical differentiation of gram-negative enteric bacilli based on the production of arginine dihydrolase and lysine and ornithine decarboxylase.

Decarboxylase Medium Base, with added arginine, lysine or ornithine is used for the same purpose.

Lysine Decarboxylase Broth is used for differentiating microorganisms based on lysine decarboxylation.

Identity Specifications

BBL™ Moeller Decarboxylase Broth Base

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.05% solution, soluble in purified water. Solution is light to medium, purple trace green to green tan purple, trace gray and rose acceptable, clear to slightly hazy.
Prepared Appearance:	Light to medium, purple trace green to green tan purple, trace gray and rose acceptable, clear to slightly hazy.
Reaction of 1.05% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

BBL™ Moeller Decarboxylase Broth Base

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C under appropriate atmospheric conditions for 4 days.

ORGANISM	ATCC™	REACTION WITHOUT LYSINE	REACTION WITH LYSINE
Enterobacter cloacae	13047	–	–
Klebsiella pneumoniae	33495	–	+

Summary and Explanation

Moeller introduced the decarboxylase media for detecting the production of lysine and 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] 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.[6]

Falkow obtained valid and reliable results with a lysine decarboxylase medium he developed to differentiate and identify *Salmonella* and *Shigella*.[9] 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,[10] substantiated the use of the medium for ornithine and arginine decarboxylase reactions as well.

Ewing, Davis and Edwards[10] 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 fomula 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.[11]

Decarboxylase tests are important in the differentiation and identification of a wide variety of microorganisms and are outlined in numerous standard methods.[12-15]

Decarboxylase Base Moeller conforms with the Moeller formulation while Decarboxylase Medium Base is prepared according to the formula described by Falkow. Lysine Decarboxylase Broth is the Falkow medium with L-lysine added in 0.5% concentration.

Principles of the Procedure

Decarboxylase basal media consist of peptones and beef or yeast extract to supply the nitrogenous and other nutrients necessary to support bacterial growth. Pyridoxal is an enzyme co-factor for the amino acid decarboxylase. 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 at a concentration of 10.0 g/L to detect the production of the enzyme specific for these substrates.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Decarboxylase Base Moeller

Dehydrated Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	1.05% solution, soluble in purified water upon boiling. Solution is yellowish-red, slightly opalescent.
Prepared Appearance:	Yellowish-red, very slightly opalescent.
Reaction of 1.05% Solution at 25°C:	pH 6.0 ± 0.2

Difco™ Decarboxylase Medium Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.9% solution, soluble in purified water upon warming. Solution is purple, clear.
Prepared Appearance:	Purple, clear.
Reaction of 0.9% Solution at 25°C:	pH 6.8 ± 0.2

Difco™ Lysine Decarboxylase Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.4% solution, soluble in purified water upon boiling. Solution is purple, clear.
Prepared Appearance:	Purple, clear.
Reaction of 1.4% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Difco™ Decarboxylase Base Moeller

Prepare the medium per label directions with and without 1% L-lysine HCl. Inoculate tubes, overlaying with sterile mineral oil, and incubate at 35 ± 2°C for 18-48 hours. Purple color indicates a positive decarboxylase reaction; a yellow color is negative.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION WITHOUT LYSINE	REACTION WITH LYSINE
Escherichia coli	25922	10^3	Good	Yellow	Purple
Shigella flexneri	12022	10^3	Good	Yellow	Yellow

Difco™ Decarboxylase Medium Base

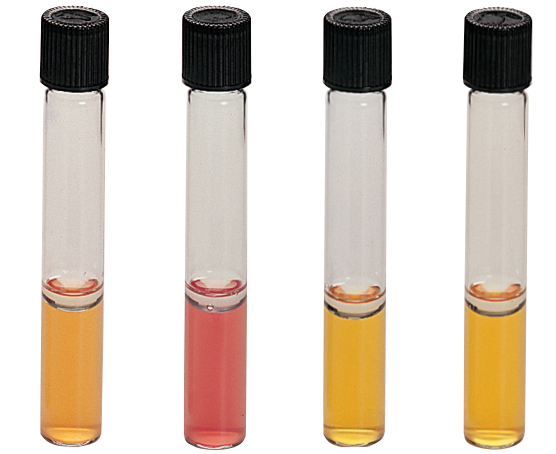
Prepare the medium per label directions. Inoculate tubes, overlaying with sterile mineral oil, and incubate at 35 ± 2°C for 40-48 hours. Purple color indicates a positive decarboxylase reaction; a yellow color is negative.

ORGANISM	ATCC™	INOCULUM CFU	REACTION WITH LYSINE	REACTION WITH ORNITHINE	REACTION WITH ARGININE
Proteus vulgaris	13315	10^3	–	–	–
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^3	+	+	+

Difco™ Lysine Decarboxylase Broth

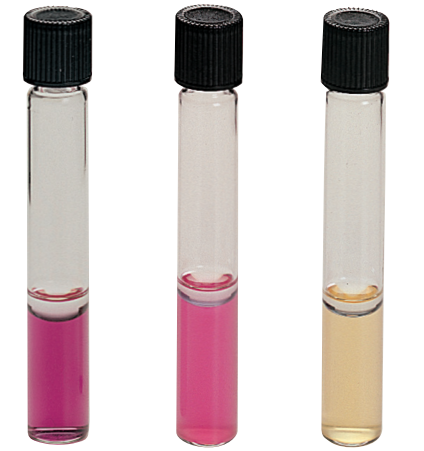
Prepare the medium per label directions. Inoculate tubes, overlaying with sterile mineral oil, and incubate at 35 ± 2°C for 18-48 hours. Purple color indicates a positive decarboxylase reaction; a yellow color is negative.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	REACTION
Escherichia coli	25922	10^3	Good	+
Proteus vulgaris	13315	10^3	Good	–



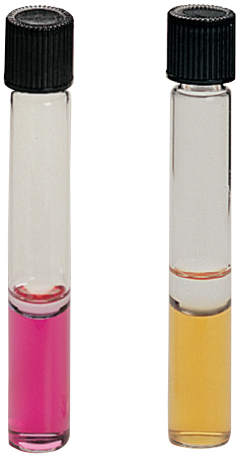
Escherichia coli ATCC™ 25922 | *E. coli* w/Lysine ATCC™ 25922 | *Shigella flexneri* ATCC™ 12022 | *S. flexneri* w/Lysine ATCC™ 12022

Decarboxylase Base Moeller



Uninoculated Tube | *Salmonella* Typhimurium ATCC™ 14028 | *Proteus vulgaris* ATCC™ 13315

Decarboxylase Medium Base



Escherichia coli ATCC™ 25922 | *Proteus vulgaris* ATCC™ 13315

Lysine Decarboxylase Broth

Continued

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 color 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 color of the indicator from yellow to purple or violet. If the organism does not produce the appropriate enzyme, the medium remains acidic (yellow). Consult the reference for more information.[16]

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.

Formulae

Difco™ Decarboxylase Base Moeller

Approximate Formula* Per Liter		
Peptone	5.0	g
Beef Extract	5.0	g
Dextrose	0.5	g
Bromcresol Purple	0.01	g
Cresol Red	5.0	mg
Pyridoxal	5.0	mg

BBL™ Moeller Decarboxylase Broth Base

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue	5.0	g
Beef Extract	5.0	g
Dextrose	0.5	g
Bromcresol Purple	0.01	g
Cresol Red	5.0	mg
Pyridoxal	5.0	mg

Difco™ Decarboxylase Medium Base

Approximate Formula* Per Liter		
Peptone	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
Bromcresol Purple	0.02	g

Difco™ Lysine Decarboxylase Broth

Approximate Formula* Per Liter		
Peptone	5.0	g
Yeast Extract	3.0	g
Dextrose	1.0	g
L-Lysine	5.0	g
Bromcresol Purple	0.02	g

** Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ Decarboxylase Base Moeller

1. Suspend 10.5 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Add 10 g of L-amino acid or 20 g of DL-amino acid and dissolve. (When adding ornithine, adjust pH using approximately 4.6 mL 1N NaOH per liter.)
4. Autoclave at 121°C for 10 minutes.
5. Test samples of the finished product for performance using stable, typical control cultures.

BBL™ Moeller Decarboxylase Broth Base

1. Suspend 10.5 g of the powder in 1 L of purified water. Add 1% of L-(or 2% of DL-)lysine, arginine or ornithine, as desired. Do not add the amino acid to the control broth.
2. Mix until a uniform suspension is obtained. Heat if necessary.
3. Autoclave at 121°C for 10 minutes. A small amount of floccular precipitate may be present in the ornithine broth, but it does not interfere with the reactions.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Decarboxylase Medium Base

1. Suspend 9 g of the powder in 1 L of purified water and warm to dissolve completely.
2. Add 5 g of L-amino acid or 10 g of DL-amino acid and warm to dissolve completely. Adjust pH if necessary.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Lysine Decarboxylase Broth

1. Suspend 14 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

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. Overlay the medium in each tube with 1 mL sterile mineral oil.

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. 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.

Expected Results

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. 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.

The medium becomes purple to violet if the reaction is positive (alkaline). A yellow color indicates a negative test; i.e., the organism does not produce the appropriate enzyme.

Limitations of the Procedure

1. If isolated or received on a selective medium, the organism should be subcultured to **Trypticase**™ Soy 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. 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.
6. A gray color may indicate reduction of the indicator. Additional indicator may be added before the results are interpreted.[12]
7. *Salmonella gallinarum* gives a delayed positive ornithine decarboxylase reaction, requiring 5-6 days incubation.[3] Many strains of *E. coli*, including those that ferment adonitol, may exhibit a delayed reaction.[3]
8. Decarboxylase Medium Base is not satisfactory for the determination of lysine decarboxylase activity with the two genera *Klebsiella* and *Enterobacter*.
9. The lysine decarboxylase activity in *Salmonella* is used to differentiate this group from *Citrobacter freundii*. *Salmonella* Paratyphi A, however, gives an atypical negative reaction (yellow color of medium) in 24 hours when Decarboxylase Medium Base is used.[4]

References

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Availability

Difco™ Decarboxylase Base Moeller

SMD SMWW USDA

Cat. No. 289020 Dehydrated – 500 g

BBL™ Moeller Decarboxylase Broth Base and Moeller Decarboxylase Broth with Amino Acids

SMD SMWW USDA

Cat. No.	211430	Dehydrated – 500 g*
	221731	Prepared Tubes, 5 mL – Pkg. of 10*
	221659	Prepared Tubes with Arginine, 5 mL – Pkg. of 10*
	221660	Prepared Tubes with Arginine, 5 mL – Ctn. of 100*
	221661	Prepared Tubes with Lysine, 5 mL – Pkg. of 10*
	221662	Prepared Tubes with Lysine, 5 mL – Ctn. of 100*
	221663	Prepared Tubes with Ornithine, 5 mL – Pkg. of 10*
	221664	Prepared Tubes with Ornithine, 5 mL – Ctn. of 100*

Difco™ Decarboxylase Medium Base

BAM CCAM COMPF ISO SMD SMWW

Cat. No. 287220 Dehydrated – 500 g

Difco™ Lysine Decarboxylase Broth

BAM CCAM COMPF ISO SMD SMWW

Cat. No. 211759 Dehydrated – 500 g

**Store at 2-8°C.*