

PRODUCT SPECIFICATION SHEET

9Lysine Decarboxylase Broth (DM136)

Intended Use

Lysine Decarboxylase Broth is used for differentiating *Salmonella* serotype *Arizonae* from the *Bethesda Ballerup* group of *Enterobacteriaceae*.

Product 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. 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. 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 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.⁽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⁾

Principles of the Procedure

During the initial stages of incubation, following inoculation, fermentation of dextrose by the organisms leads to acid production, which causes a subsequent colour change of the bromocresol purple indicator to yellow. The acidic condition thus generated stimulates decarboxylase activity, which leads to decarboxylation of lysine to cadaverine. The alkaline conditions generated due to cadaverine production cause the bromocresol purple indicator (changed to yellow) to revert to purple colour. If the organisms do not produce decarboxylase enzyme, the colour of the medium remains yellow. Dextrose non-utilizers will not show any change in the medium colour. Use light inocula and do not read the tests after 24 hours incubation, as some organisms require longer incubation time of up to 4 days. Lysine decarboxylase medium consists of peptic digest of animal tissue and yeast extract to supply the nitrogenous and other nutrients necessary to support bacterial growth. Dextrose is a fermentable carbohydrate. L-Lysine hydrochloride is the amino acid and Bromocresol purple is the pH indicator.

Formula / Liter

| Ingredients | Gms / Liter |
|--|-------------|
| Peptic digest of animal tissue | 5.00 |
| Yeast extract | 3.00 |
| Dextrose | 1.00 |
| L-Lysine hydrochloride | 5.00 |
| Bromocresol purple | 0.02 |
| Final pH: 6.8±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.

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Directions

1. Suspend 14.02 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely, with frequent agitation to avoid charring.
3. Dispense 5 ml amount into screw-capped test tubes.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control Specifications

| | |
|----------------------------------|---|
| Dehydrated Appearance | Light yellow to greenish yellow homogeneous free flowing powder |
| Prepared Medium | Purple coloured clear solution without any precipitate. |
| Reaction of 1.4% solution | pH 6.8±0.2 at 25°C |
| Gel Strength | Not Applicable |

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. (Inoculated tubes are overlaid with sterile mineral oil).

| Sr. No. | Organisms | Results to be achieved | |
|---------|--|------------------------|----------------------------------|
| | | Inoculum (CFU) | Lysine decarboxylation |
| 1. | <i>Citrobacter freundii</i> ATCC 8090 | 50-100 | Variable reaction |
| 2. | <i>Escherichia coli</i> ATCC 25922 | 50-100 | Variable reaction |
| 3. | <i>Enterobacter aerogenes</i> ATCC 13048 | 50-100 | Positive reaction, purple colour |
| 4. | <i>Klebsiella pneumonia</i> ATCC 13883 | 50-100 | Positive reaction, purple colour |
| 5. | <i>Proteus mirabilis</i> ATCC 25933 | 50-100 | Negative reaction, yellow colour |
| 6. | <i>Proteus vulgaris</i> ATCC 13315 | 50-100 | Negative reaction, yellow colour |
| 7. | <i>Salmonella Arizonae</i> ATCC 13314 | 50-100 | Positive reaction, purple colour |
| 8. | <i>Salmonella Paratyphi A</i> ATCC 9150 | 50-100 | Negative reaction, yellow colour |
| 9. | <i>Salmonella Typhi</i> ATCC 6539 | 50-100 | Positive reaction, purple colour |
| 10. | <i>Serratia marcescens</i> ATCC 8100 | 50-100 | Positive reaction, purple colour |
| 11. | <i>Shigella dysenteriae</i> ATCC 13313 | 50-100 | 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 ± 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.

Results

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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). 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 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

Use light inocula for the tubes of lysine medium. Note the negative reaction of *Salmonella paratyphi* A. Do not read the test under 24 hours incubation. Some organisms may require prolonged incubation, up to 4 days.

Packaging

Product Name : Lysine Decarboxylase Broth

Product Code : DM 136

Available Pack sizes : 100gm / 500gm

References

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Further Information

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