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Lysine Decarboxylase Broth w/o Peptone (DM730)

Intended Use

Lysine Decarboxylase Broth w/o Peptone (DM730) is recommended for ISO Committee for distinguishing the *Salmonella* serotype *Arizonae* from the *Bethesda Ballerup* group of *Enterobacteriaceae*.

Product Summary and Explanation

In 1955, Moeller formulated the decarboxylase media for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase. [1-3] Prior to Moellers work, bacterial amino acid decarboxylases were studied by Gale [4] and Gale and Epps. [5] Many species of bacteria possess enzymes capable of decarboxylating specific amino acids in the test medium releasing alkaline-reacting amines and carbon dioxide as byproducts. The decarboxylase activity of *Enterobacteriaceae* is most commonly measured with Moeller Decarboxylase Broth. [1]

Lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* was developed by Falkow.[6] Falkow's Medium was further modified by Taylor[7] by deleting peptone from the formulation (DM730), thus eliminating false positives caused by *Citrobacter freundii* and its paracolons. Taylor's modification has same advantage of Falkow's formulation over Moeller; it does not require the special conditions of anaerobic culture and low pH.

Principles of the Procedure

Lysine Decarboxylase Broth w/o Peptone contains yeast extract which provide nitrogenous nutrients necessary for the growth of bacteria. Dextrose is the fermentable carbohydrate. Bromo cresol purple is the pH indicators in this medium. The amino acid L-lysine hydrochloride is added to the basal medium to detect the production of the enzyme specific for these substrates.

Formula / Liter

Ingredients	Gms / Liter
L-Lysine hydrochloride	5.00
Yeast extract	3.00
Dextrose	1.00
Bromocresol purple	0.015
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.

Directions

1. Suspend 9.01 grams of the medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense 5 ml amount into screw-capped test tubes.
4. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
5. Cool the tubed medum in an upright position and overlay with 2-3 ml of sterile mineral oil.

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 0.9% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Not Applicable

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Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Lysine Decarboxylation
1.	*Citrobacter freundii ATCC 8090*	50 – 100	variable reaction
2.	*Escherichia coli ATCC 25922*	50 – 100	variable reaction
3.	*Enterobacter aerogenes ATCC 13048*	50 – 100	positive reaction, purple colour
4.	*Klebsiella pneumonia ATCC 13883*	50 – 100	positive reaction, purple colour
5.	*Proteus mirabilis ATCC 25933*	50 – 100	negative reaction, yellow colour
6.	*Proteus vulgaris ATCC 13315*	50 – 100	negative reaction, yellow colour
7.	*Salmonella Arizonae ATCC13314*	50 – 100	positive reaction, purple colour
8.	*Salmonella Paratyphi A ATCC 9150*	50 – 100	negative reaction, yellow colour
9.	*Salmonella Typhi ATCC 6539*	50 – 100	positive reaction, purple colour
10.	*Serratia marcescens ATCC 8100*	50 – 100	positive reaction, purple colour
11.	*Shigella dysenteriae 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 25 grams of the test sample into Buffered Peptone Water (DM049).
2. After incubation at 35-37°C for 16-20 hours, inoculate into RVS Broth (DM1405) and Fluid Selenite Cystine Medium (DM475) and incubate at 35-37°C for 24-48 hours.
3. From the second enrichment, streak a loopful on Brilliant Green Agar Base w/ phosphates (DM717).
4. Presumptive *Salmonella* so isolated on Brilliant Green Agar Base w/ phosphates (DM717) are further confirmed by performing biochemical testing using the following media i.e. Nutrient Agar, pH 7.0 (DM1805), Triple Sugar Iron Agar (DM245), Urea Agar Base, Lysine Decarboxylase Broth w/o peptone (DM730), VP test, Indole test.

Results

1. Fermentation of dextrose by the organisms, with acid production results in a colour change of the indicator to yellow.
2. On further incubation, if L-Lysine is decarboxylated to cadaverine, there will be an alkaline reaction and the indicator colour will then revert back to purple.
3. If the colour remains yellow, the decarboxylase reaction is negative.

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.

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Limitations of the Procedure

1. Use light inocula and do not read the tests under 24 hours incubation as some organisms require longer incubation time of upto 4 days.
2. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Lysine Decarboxylase Broth w/o Peptone
Product Code : DM730
Available Pack sizes : 100gm/ 500gm

References

1. Moeller, V. 1955. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta. Pathol. Microbiol. Scand. *36*:158-172.
2. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:102.
3. Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:259.
4. Gale G. F., 1940, Biochem. J., 34:392.
5. Gale and Epps, 1943, Nature, 152:327.
6. Falkow, 1958, Am. J. Clin. Pathol., 29:598.
7. Taylor W. I., 1961, Appl. Microbiol., 9:487.

Further Information

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