IFU

Instructions for Use

MOELLERS DECARBOXYLASE MEDIA

Cat. no. Y41	Moellers Decarboxylase Base, 16x125mm Tube, 5ml	20 or 100 tubes/box
Cat. no. Y42	Moellers Arginine Decarboxylase, 16x125mm Tube, 5ml	20 or 100 tubes/box
Cat. no. Y43	Moellers Lysine Decarboxylase, 16x125mm Tube, 5ml	20 or 100 tubes/box
Cat. no. Y44	Moellers Ornithine Decarboxylase, 16x125mm Tube, 5ml	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics Moellers Decarboxylase Media is recommended for the differentiation of gram-negative enteric bacilli based on the production of arginine dihydrolase, lysine decarboxylase or ornithine decarboxylase.

SUMMARY

Hardy Diagnostics Moellers Decarboxylase Media employs the formula established by Moeller, who, in 1955, first introduced its use in the differentiation of gram-negative enteric bacilli.[6] The basal media consists of peptones, beef extract, pyridoxal, glucose, bromcresol purple and cresol red. Necessary growth nutrients are supplied by the peptones and beef extract. Pyridoxal is an enzyme co-factor. Bromcresol purple and cresol red serve as pH indicators that aid in the detection of glucose-fermentation and amino acid decarboxylation. Arginine, lysine or ornithine are added to the basal medium to detect the production of specific amino acid decarboxylase and dihydrolase enzymes.

The production of decarboxylase and dihydrolase enzymes is induced in an acidic environment. In the case of Moeller Decarboxylase Media, an acidic state is established when glucose is fermented by the microorganism resulting in a color shift from purple to yellow. Microorganisms possessing the specific decarboxylase and dihydrolase enzymes for the amino acid (arginine, lysine or ornithine) degrade the amino acid to yield various amine by-products. An alkaline environment is thereby established. The increased pH results in a color shift from the previous yellow to a purple or gray-purple. If the organism does not produce the appropriate enzyme, then the medium will retain the yellow color indicative of glucose-fermentation. Non-glucose-fermenters that possess decarboxylase and dihydrolase enzymes may display weak decarboxylase activity to produce little or no color change as compared to an uninoculated tube of basal medium.

FORMULA

Ingredients per liter of deionized water:*

Moellers Decarboxylase Base:	
Peptone	5.0gm
Beef Extract	5.0gm
Glucose	0.5gm

Bromcresol Purple	10.0mg
Cresol Red	5.0mg
Pyridoxal	5.0mg

Additionally,

Moellers Arginine Decarboxylase contains:	
Arginine	10.0gm

Moellers Lysine Decarboxylase contains:	
DL-Lysine	20.0gm

Moellers Ornithine Decarboxylase contains:	
Ornithine	10.0gm

Final pH 6.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Specimen collection is not applicable since this medium is not intended for primary isolation from clinical specimens. As a general rule, infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on specimen collection.[1-5]

Method of Use:

1. Inoculate the media using 1-2 isolated colonies taken from an 18-24 hour pure culture.

2. Inoculate a control tube of Decarboxylase Base (Cat. no. Y41) in parallel with the amino acid based media.

3. Overlay each inoculated tube with 1ml sterile mineral oil (Cat. no. Z80).

4. Tighten the caps on the inoculated tubes and incubate aerobically at 35ºC. for 18-96 hours.
Note: Increased incubation for up to 10 days may be necessary for some microorganisms.

5. Observe daily for color reactions. Compare results with the inoculated control tube.

INTERPRETATION OF RESULTS

A positive decarboxylase result is indicated by the development of a purple to pale yellow-purple color.

A negative decarboxylase result is indicated by the development of a bright yellow color for glucose-fermenting microorganisms. Non-glucose-fermenters will result in little or no color change as compared to an uninoculated tube.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Mineral oil must be applied to the surface of each inoculated medium. Oil overlay decreases the possibility of an alkaline shift occurring in the medium due to oxidation.

Test interpretation should **not** be made prior to 18-24 hours of incubation. Earlier interpretation may lead to erroneous results. Glucose-fermentation occurs within the first 10-12 hours of incubation. Fermentation produces an acidic environment which results in a yellow color development. The production of decarboxylase and dihydrolase enzymes will not be induced until the acidic state has been established.

Decarboxylation results for non-glucose-fermenting microorganisms may prove unreliable. This test relies on the inducement of decarboxylase and dihydrolase enzymes by acid produced from glucose-fermentation. Decarboxylation results for non-glucose-fermenting microorganisms, therefore, may display weak decarboxylase activity thereby resulting in an insufficient production of amines necessary to convert the pH indicator system. Some non-fermenters, however, will produce sufficient amines and result in a deeper purple color as compared to an uninoculated tube of basal medium.

Non-glucose-fermenting microorganisms that do not produce the appropriate enzyme remain the same color as the original uninoculated control tube of basal medium.

Organisms that do not produce the appropriate enzyme, but do utilize glucose, will result in a yellow color development in the medium.

Development of a purple (alkaline) color in the uninoculated control tube of basal medium invalidates all test results, and test interpretation should not be made.

Increased incubation for up to 10 days may be necessary for some microorganisms.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, mineral oil, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Klebsiella pneumoniae ATCC® 13883	E**	24-96hr	35°C	Aerobic	Growth; Arginine: negative, Lysine: positive, Ornithine: negative
Enterobacter cloacae ATCC® 23355	E**	24-96hr	35°C	Aerobic	Growth; Arginine: positive, Lysine: negative, Ornithine: positive
Proteus mirabilis ATCC® 12453	E**	24-96hr	35°C	Aerobic	Growth; Arginine: negative, Lysine: negative, Ornithine: positive

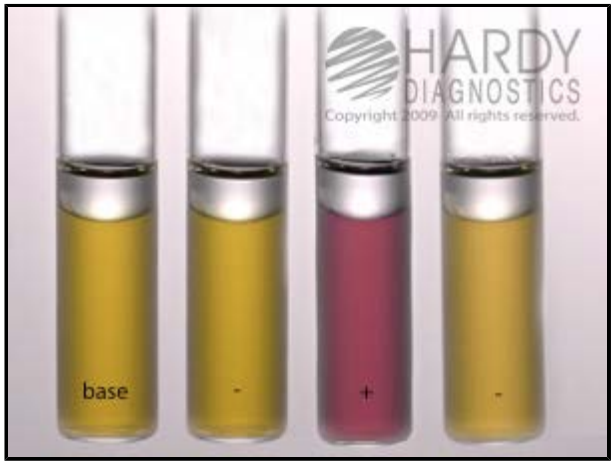
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

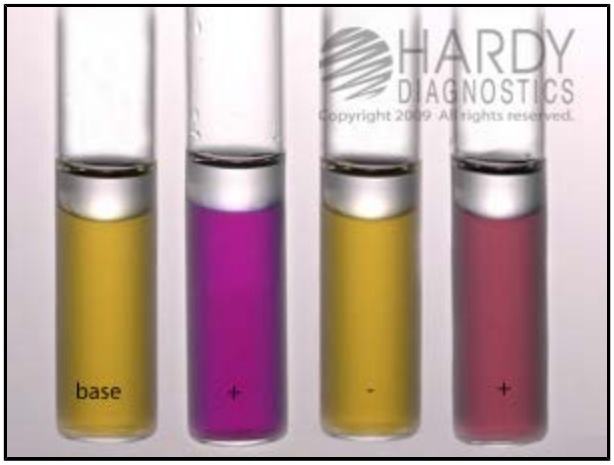
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

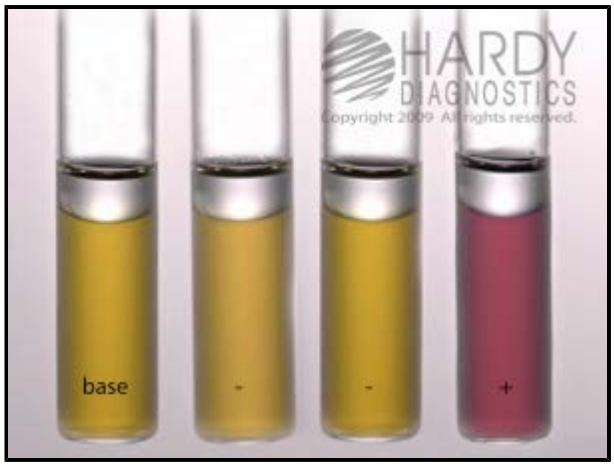
Moellers Decarboxylase Media should appear clear, slightly opalescent, and yellowish-red in color.



Klebsiella pneumoniae (ATCC® 13883) growing in Moellers Decarboxylase Media. Incubated aerobically with sterile mineral oil (Cat. no. Z80) layer and tightened caps for 24 hours at 35ºC. **Pictured:** Moellers Base (Cat. no. Y41), Moellers Arginine (Cat. no. Y42), Moellers Lysine (Cat. no. Y43), Moellers Ornithine (Cat. no. Y44).



Enterobacter cloacae (ATCC® 23355) growing in Moellers Decaboxylase Media. Incubated aerobically with sterile mineral oil layer (Cat. no. Z80) and tightened caps for 24 hours at 35ºC. **Pictured:** Moellers Base (Cat. no. Y41), Moellers Arginine (Cat. no. Y42), Moellers Lysine (Cat. no. Y43), Moellers Ornithine (Cat. no. Y44).



Proteus mirabilis (ATCC® 12453) growing in Moellers Decaboxylase Media. Incubated aerobically with sterile mineral oil layer (Cat. no. Z80) and tightened caps for 24 hours at 35ºC. **Pictured:** Moellers Base (Cat. no. Y41), Moellers Arginine (Cat. no. Y42), Moellers Lysine (Cat. no. Y43), Moellers Ornithine (Cat. no. Y44).

Uninoculated tube of Moellers Decaboxylase Base (Cat. no. Y41).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Moeller. 1955. *Acta Pathol. MIcrobiol. Scand.*; 36:158.

IFU-10585[B]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]