IFU

Instructions for Use

NOCARDIA HYDROLYSIS KIT

Cat. no. Z604	Nocardia Hydrolysis Kit	20 tubes/kit
Contains five tubes each of the following formulas:		
Cat. no. Q60A	Casein Agar, 20x125mm Tube, 18ml Deep	
Cat. no. Q60B	Xanthine, 20x125mm Tube, 18ml Deep	
Cat. no. Q60C	Hypoxanthine, 20x125mm Tube, 18ml Deep	
Cat. no. Q60D	Tyrosine, 20x125mm Tube, 18ml Deep	

INTENDED USE

Hardy Diagnostics Nocardia Hydrolysis Kit is designed to aid in the differentiation of aerobic *Actinomycetes*, *Nocardia*, *Actinomadura*, and *Streptomyces* species by determining the presence of hydrolysis enzymes within these species.

SUMMARY

These agars are useful in differentiating *Nocardia* , *Actinomadura* , and *Streptomyces* species on the basis of their patterns of hydrolysis. Certain species of aerobic *Actinomycetes* produce hydrolytic enzymes that degrade proteins, purines and amino acids. If hydrolysis occurs, a clearing of the media takes place.

FORMULA

Ingredients per liter of deionized water:*

Casein Agar (Q60A):	
Skim Milk	75.0gm
Agar	15.0gm
Xanthine Agar (Q60B):	
Pancreatic Digest of Gelatin	5.0gm
Xanthine	4.0gm
Beef Extract	3.0gm
Agar	15.0gm
Hypoxanthine Agar (Q60C):	
Hypoxanthine	5.0gm

Pancreatic Digest of Gelatin	5.0gm
Beef Extract	3.0gm
Agar	15.0gm
Tyrosine Agar (Q60D):	
Tyrosine	5.0gm
Pancreatic Digest of Gelatin	5.0gm
Beef Extract	3.0gm
Agar	15.0gm

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. in the dark. Media should not be used if there are any signs of deterioration (shrinking, cracking, or 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: Not applicable since these media are not primary isolation. These media are used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating these media. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.[3,4,6]

Place the tubes in a boiling waterbath to melt agar.

After agar has melted, allow to cool to just short of solidification, and pour into chilled (2-8ºC.) 15x100mm petri dishes.

Inoculate by streaking or spot inoculating (about the size of a quarter) each plate with a pure suspension of previously isolated organisms. Incubate at 25ºC. (or 35ºC. if necessary) for up to 3 weeks. Examine each section for growth and clearing or hydrolysis at 7, 14, and 21 days.

Expected Results:				
Organism	**Casein**	**Tyrosine**	**Xanthine**	**Hypoxanthine**
Nocardia otitidiscaviarum (*caviae*)	-	-	+	+
Nocardia farcinica	-	-	-	-
Nocardia brasiliensis	+	+	-	+

INTERPRETATION OF RESULTS

Positive Test - Clearing is observed around and/or beneath colony growth (hydrolysis).

Negative Test - No clearing is observed around and/or beneath the inoculum.

LIMITATIONS

It is essential that the tyrosine and xanthine be kept in suspension while the media solidifies. After boiling the agar deeps, allow the molten agar to cool just short of solidification. Refrigerate the petri dishes and remove just prior to pouring. Mix the agar deeps thoroughly, and distribute evenly into the cold plate.

It is essential that a control organism be placed on each plate to determine the efficacy of the media.

Media should be freshly prepared for optimum reactions.

All crystals should dissolve completely before pouring into the plate.

Sealing the plates may be necessary to reduce dehydration and possible aerial dissemination of hyphal structures (MycoSeal™ Cat. no. SS9225).

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, MycoSeal™ (Cat. no. SS9225), 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:

	Inoculation	**Incubation**	

Test Organisms	Method*	Time	Temperature	Atmosphere	Results
Nocardia brasiliensis ATCC® 19296	G	2 weeks	15-30°C	Aerobic	Growth on all and positive for casein, hypoxanthine and tyrosine hydrolysis
Nocardia otitidiscaviarum (*caviae*) ATCC® 14629	G	2 weeks	15-30°C	Aerobic	Growth on all and positive for xanthine and hypoxanthine hydrolysis
Nocardia farcinica ATCC® 3308	G	2 weeks	15-30°C	Aerobic	Growth on all and negative for casein, xanthine, hypoxanthine and tyrosine hydrolysis

* 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

- Casein Agar should appear opaque, with precipitate; and white to off-white in color.
- Xanthine Agar should appear opaque, with precipitate; and cream to white in color.
- Hypoxanthine Agar should appear opaque, with precipitate; and cream to white in color.
- Tyrosine Agar products appear opaque, with precipitate; and cream to white in color.

Nocardia otitidiscaviarum(ATCC® 14629) growing on Hypoxanthine Agar (Cat. no. Q60C) from the Nocardia Hydrolysis Kit (Cat. no. Z604). Showing positive growth, positive hydrolysis. Incubated aerobically for 2 weeks at 30ºC.

REFERENCES

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

4. Lennette, E.H., et al. 1985. *Manual of Clinical Microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.

5. Balows, A., K.L. Hermann, H. Isenberg, H.J. Shadomy, and W.J. Hausler, Jr. 1991. *Manual of Clinical Microbiology*, 5th ed. ASM, Washington, D.C.

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8. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

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