IFU

Instructions for Use

UREA R BROTH

Cat. no. Y141	Urea R Broth, 13x100mm Tube, 1ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Urea R Broth is a highly sensitive medium used for the rapid detection of urease activity by microorganisms.

SUMMARY

The principle of the urease test is to determine whether an organism has the ability to hydrolyze urea into two molecules of ammonia by the enzyme urease (urea amidohydrolase) which results in increased alkalinity of the medium. This increase in alkalinity is detected by the use of phenol red as the pH indicator, changing from a orange-red to a bright pink.[1]

Urea R Broth is designed to be both rapid and sensitive for the detection of urease. Urea R Broth utilizes weak pH buffers monopotassium phosphate and disodium phosphate in very low concentrations in order to detect the smallest amount of alkali. Yeast extract acts as a source of nutrients, carbon, and nitrogen for those organisms incapable of using ammonia as a nitrogen source.[1]

There are several different formulas for determining the urease reaction of a microorganism. For the strong urease producers like *Proteus* spp. there is Rustigian and Stuart's Urea Broth. For the weak urease producers such as *Klebsiella pneumoniae* and *Morganella morganii* there is Urea R Broth.[1] *Staphylococcus epidermidis* , *S. intermedius* and most strains of *S. saprophyticus* are usually urease-positive using Urea R Broth.[5] In addition to these organisms, Urea R Broth aids in the identification of certain yeast and filamentous fungi. *Cryptococcus* spp. and *Rhodotorula* spp. are urease-positive. Most strains of *Trichosporon* spp. are urease-positive and *Geotrichum* spp. and *Blastoschizomyces capitatus* are urease-negative.[4,5] Almost all strains of *Candida* spp. found in clinical specimens are urease-negative, with the exception of *C. lipolytica* and some strains of *C. krusei* which are urease-positive.[5] Several species of *Trichophyton* are urease-positive when tested with Urea R Broth.

FORMULA

Ingredients per liter of deionized water:*

Urea	20.0gm
Yeast Extract	0.1gm
Disodium Phosphate	0.095gm
Monopotassium Phosphate	0.091gm
Phenol Red	0.01gm

Final pH 6.9 +/- 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. 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: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Method of Use:

Using a sterile loop, pick up three to four isolated colonies from a pure culture to inoculate the broth. Incubate aerobically at 35ºC. Check for color change at 6 to 8 and 12 to18 hours.

When testing *Trichophyton* spp. for urease, most reactions will become positive after 48-72 hours, but if still negative, hold for a maximum of 7 days before determining the test negative.[3]

INTERPRETATION OF RESULTS

A positive urease reaction is indicated by a color change from a reddish-orange to a bright pink. A negative reaction is indicated by no color change. It is often helpful to use an uninoculated tube as a negative control.[1] Consult listed references for the identification of colony morphology and further biochemical tests required for identification.[1,2,4,5]

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.

If not stored at 2-8ºC., urea has been shown to undergo autohydrolysis, which could interfere with the urease reaction.(1)

Plastic tubes or wells or tubes made of borosilicate type I glass is recommended to prevent non-specific alkaline shifts due to other glass types.

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, 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	
Proteus hauseri ATCC® 13315	E	12-18hr	35°C	Aerobic	Positive; color change to bright pink
Escherichia coli ATCC® 25922	E	12-18hr	35°C	Aerobic	Negative; no color change

* 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

Urea R Broth should appear clear, and reddish-orange in color.

Proteus hauseri (ATCC® 13315) growing in Urea R Broth (Cat. no. Y141). The bright pink color change was indicative as positive for urease hydrolysis. Incubated aerobically for 18 hours at 35ºC.

Escherichia coli (ATCC® 25922) growing in Urea R Broth (Cat. no. Y141). The absence of a pink color change was indicative as negative for urease hydrolysis. Incubated aerobically for 18 hours at 35ºC.

REFERENCES

1. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria,*, Lipincott Williams & Wilkins, Philadelphia, PA.

2. Baron, E.J, Peterson, L.R., and Finegold, S.M. 1994. *Bailey and Scott's Diagnostic Microbiology*, 9th ed. Mosby, St. Louis, MO.

3. Sutton, D.A., Fothergill, A.W., and Rinaldi, M.G. *Guide to Clinically Significant Fungi*. Williams & Wilkins.

4. St. Germain, Guy, et al. 1996. *Identifying Filamentous Fungi*. Star Publishing Company, Belmont, CA.

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

6. Larone, D.H. *Medically Important Fungi: A Guide to Identification*, American Society for Microbiology. Washington, D.C.

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The Hardy Diagnostics manufacturing facility and quality

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