

# Instructions for Use

## UREA AGAR SLANT

<a href="#">Cat. no. L65</a>	Urea Agar, 16x100mm Tube, 5.5ml Slant	20 or 100 tubes/box
<a href="#">Cat. no. R42</a>	Urea Agar, 13x100mm Tube, 3ml Slant	20 or 100 tubes/box

## INTENDED USE

Hardy Diagnostics Urea Agar is recommended for use in the detection of urea hydrolysis in gram-negative organisms.

## SUMMARY

Urea Agar was developed by Christensen in 1946 for the differentiation of enteric bacilli, as an improvement over the broth method used at the time.<sup>(3)</sup> Urea Agar is used to differentiate between rapidly positive *Proteus* species and other slower urea positive members of the Enterobacteriaceae. This medium may also be used in the detection of urease activity in other gram-negative organisms, such as *Pseudomonas*, *Pasteurella*, and *Brucella*.<sup>(5)</sup> Webb, et al. also reported that Urea Agar is useful in differentiating *Cryptococcus* from other yeast species.<sup>(9)</sup>

Urea Agar contains urea and phenol red as the pH indicator. Organisms capable of hydrolyzing urea form ammonia as a by product, thus turning the medium alkaline. The pH indicator turns from pale yellow to pink-red in color in these conditions. The reduced buffer content and peptone in this medium promote more rapid growth and reaction time for many members of the Enterobacteriaceae. Dextrose is included in the formulation to stimulate urease activity in organisms that hydrolyze urea slowly, and to exclude false-negative reactions. *Proteus* species rapidly hydrolyze urea, and a positive reaction is usually seen within one to six hours. Other organisms perform this reaction slower, and may require a 24 to 48 hour, or longer, incubation time.

## FORMULA

Ingredients per liter of deionized water:\*

Urea	20.0gm
Sodium Chloride	5.0gm
Monopotassium Phosphate	2.0gm
Peptone	1.0gm
Dextrose	1.0gm
Phenol Red	0.012gm
Agar	15.0gm

Final pH 6.7 +/- 0.2 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. 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: 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: Allow Urea Agar to warm to room temperature before use. Using a sterile loop, pick up three to four isolated colonies from a pure culture. Streak back and forth over the surface of the slant. Do not stab the butt, as it serves as a color control. Incubate aerobically at 35°C. with a loosened cap. Examine after 2, 6, and 24 hours for results. If a positive reaction is not seen after this time, examine daily for up to six days to detect organisms that hydrolyze urea slowly.

## INTERPRETATION OF RESULTS

A positive urease reaction is indicated by a color change to red-pink. Consult listed references for the identification of colony morphology and further biochemical tests required for identification.<sup>(1,2,4,7)</sup>

## 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.

To facilitate growth and the urea hydrolysis reaction, do not use inoculum from a broth suspension.

After prolonged incubation times a false-positive alkaline reaction may be seen. To rule out this occurrence, check the test with a control (an uninoculated tube of Urea Agar) along with the inoculated tube during prolonged incubation.

Do not heat the Urea Agar Slants, as urea decomposes very readily when heated.

To detect *Proteus* species, the Urea Agar, Slants must be examined within 6 hours of inoculation for a reaction.

Urea Agar should not be used to determine the quantitative rate of urease activity, as organisms vary in their capability and rate of hydrolysis.

Failure to incubate this medium with loose caps may cause erroneous results to occur.

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	
<i>Proteus mirabilis</i> ATCC® 12453	E	18-24hr	35°C	Aerobic	Positive: growth; pink color change
<i>Escherichia coli</i> ATCC® 25922	E	18-24hr	35°C	Aerobic	Negative: growth; yellow color change

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

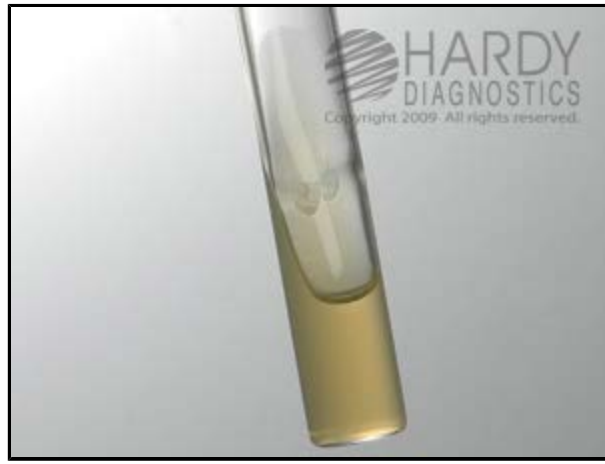
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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 Agar, Slants should appear opalescent, and light orange in color.



*Proteus mirabilis* ( ATCC® 12453) growing on Urea Agar (Cat. no. R42). The pink color change was indicative as positive for urea hydrolysis. Incubated aerobically for 24 hours at 35°C.



*Escherichia coli* ( ATCC® 25922) growing on Urea Agar (Cat. no. R42). The absence of a pink color change was indicative as negative for urea hydrolysis. Incubated aerobically for 24 hours at 35°C.

## 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.
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8. *Quality Assurance for Commercially Prepared Microbiological Culture Media, M22*. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
9. Webb, C.D., et al. 1973. *Identification of Yeasts*, U.S.D.H.E.W., CDC, Atlanta, GA.

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