BBL™ Urease Broth Concentrate 10X
BBL™ Urease Test Broth, 3 mL

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Urease Test Broth is a differential medium for organisms, particularly members of the *Enterobacteriaceae*, on the basis of their ability to produce urease.

II PERFORMANCE TEST PROCEDURE

A. Directions for preparation of a complete medium from Urease Broth Concentrate 10X.
   1. To prepare the medium, aseptically add 1 mL of the concentrate to 9 mL of cold sterile purified water. Mix thoroughly.
   2. Dispense aseptically in 3 mL amounts, in small sterile test tubes.

B. Testing of complete medium (Urease Test Broth)
   1. Inoculate representative samples with the cultures listed below.
      a. Using a 0.01 mL calibrated loop, inoculate the broth with heavy (3 loopfuls) inocula using 24- to 48-h **Trypticase™** Soy Agar Slant cultures.
      b. Incubate tubes with loosened caps at 35 ± 2 °C (incubator or water bath) in an aerobic atmosphere.
   2. Examine tubes after 2, 4, 6 and 24 h for growth and reactions.
   3. Expected Results

Organisms	ATCC™	Urease Reaction
**Proteus vulgaris*	8427	+ (Intense pink-red to red-violet color)
Morganella morganii subsp. *morganii*	8019	+ (Intense pink-red to red-violet color)
**Salmonella enterica subsp. enterica serotype Typhimurium*	13311	– (No color change)

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.8 ± 0.2.
4. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Urease Test Broth is used for the differentiation of organisms, especially the *Enterobacteriaceae*, on the basis of urease production.

V SUMMARY AND EXPLANATION

Urease Test Broth was developed by Rustigian and Stuart.[1] It may be used for the identification of bacteria on the basis of urea utilization and it is particularly recommended for the differentiation of members of the genus *Proteus* from those of *Salmonella* and *Shigella* in the diagnosis of enteric infections.[2] The medium is positive for *Proteus*, *Morganella morganii* subsp. *morganii, Providencia rettgeri*, and a few *Providencia stuartii* strains with the reclassification of the members of the *Proteeae*.

Urease base is also supplied as a filter sterilized 10X concentrated solution for use in preparing Urease Test Broth in the user’s laboratory.

VI PRINCIPLES OF THE PROCEDURE

The urea medium of Rustigian and Stuart[1] is particularly suited for the differentiation of *Proteus* species from other gram-negative enteric bacilli capable of utilizing urea,[3] the latter being unable to do so in Urease Test Broth because of limited nutrients and the high buffering capacity of the medium.

When organisms utilize urea, ammonia is formed during incubation which makes the reaction of these media alkaline, producing a pink-red color. Consequently, urease production may be detected by the change in the phenol red indicator.

VII REAGENTS

Urease Broth Concentrate 10X

Approximate Formula* Per Liter Purified Water

Urea	200.0 g	Yeast Extract	1.0 g
Monopotassium Phosphate	91.0 g	Phenol Red	0.1 g
Disodium Phosphate	95.0 g		

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

Urease Test Broth

Approximate Formula* Per Liter Purified Water

Urea	20.0 g	Yeast Extract	0.1 g
Monopotassium Phosphate	9.1 g	Phenol Red	0.01 g
Disodium Phosphate	9.5 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Minimize exposure to light.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[2,4] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Urease Test Broth or Urease Broth Concentrate, 10X

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

If the Urease Broth Concentrate 10X is being used, prepare the complete medium as described in the Quality Control section. If crystals form in the concentrate, they will usually dissolve at room temperature, or in a few minutes in a 40 °C water bath.

Using a heavy inoculum (3 loopfuls) of growth from an 18- to 24-h pure culture (TSI Agar or other suitable medium), inoculate the broth. Shake the tubes gently to suspend the bacteria. Incubate tubes with loosened caps at 35 ± 2 °C in an incubator or water bath. Observe reactions after 2, 4, 6, 24 and 48 h.

User Quality Control: See “Quality Control Procedures.”

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory’s standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

The production of urease is indicated by an intense pink-red (red-violet) color throughout the broth.

A negative reaction is no color change: the broth remains yellowish-orange.

For a listing of urease-positive organisms, consult appropriate texts.[2,5,6]

XI LIMITATIONS OF THE PROCEDURE

All urea test media rely on the demonstration of alkalinity; hence, they are not specific for urease. The utilization of peptones (e.g., by *Pseudomonas aeruginosa*) or other proteins in the medium may raise the pH to alkalinity due to protein hydrolysis and release of excessive amino acid residues, resulting in false-positive reactions.[7]

For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[2,4-6]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Urease Test broth are tested for performance characteristics. Using a 0.01 mL calibrated loop, representative samples of the lot are inoculated with three loopfuls of **Trypticase** Soy Agar cultures of *Morganella morganii* (ATCC 8019), *Proteus vulgaris* (ATCC 8427), and *Salmonella* Typhimurium (ATCC 13311). Inoculated tubes with loosened caps are incubated at 35 ± 2 °C and read at 2, 4, 6, 24, and 48 h for reactions. *M. morganii* and *P. vulgaris* produce a rose red color in the medium within 4 h indicating the formation of ammonia, from urea utilization, thus rendering the medium alkaline. *Salmonella* Typhimurium is negative for urease production and no color change is evident in the medium.

XIII AVAILABILITY

Cat. No.	Description
221719	**BBL™** Urease Test Broth, 3 mL, Pkg. of 10 size K tubes
221098	**BBL™** Urease Broth Concentrate, 10X, Pkg. of 10 size K tubes

XIV REFERENCES

1. Rustigian, R., and C.A. Stuart. 1941. Decomposition of urea by *Proteus*. Proc. Soc. Exp. Biol. Med. *47*:108-112.
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3. Christensen, W.B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bacteriol. *52*:461-466.
4. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
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6. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. MacFaddin, J.F. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott, Williams & Wilkins, Baltimore.

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