UREA AGAR BASE (7226)

Intended Use
Urea Agar Base is used with agar for the differentiation of microorganisms on the basis of urease production.

Product Summary and Explanation
Christensen devised a urea agar medium containing peptone and dextrose that had a reduced buffer content.[1] The medium supported a vigorous growth of many Gram-negative, enteric bacilli and readily permitted observation of urease production. Ewing used Urea Agar Base as a differential medium in the examination of many cultures from stool specimens.[2] Urea Agar Base may be used as a screening medium (along with Triple Sugar Iron Agar) for the selection of *Salmonella* and *Shigella* cultures for serologic classification.[3] Urea Agar Base is used to detect production of urease by yeast.[4] Urease production is an important differential test in microbiology and outlined in standard methods.[5-7]

Principles of the Procedure
Enzymatic Digest of Gelatin provides nitrogen, carbon, and amino acids required for organism growth in Urea Agar Base. Dextrose is an energy source. Sodium Chloride maintains the osmotic balance of the medium. Monopotassium Phosphate is the buffer. Urea provides a nitrogen source for organisms producing urease. The splitting of urea by urease causes the release of ammonia, increasing pH of the medium to the alkaline side. This is indicated by a color change of the pH indicator, Phenol Red, from yellow (pH 6.8) to red (pH 8.1). Agar is added as a supplement to solidify the medium.

Formula / Liter
Enzymatic Digest of Gelatin	1 g
Dextrose	1 g
Sodium Chloride	5 g
Monopotassium Phosphate	2 g
Urea	20 g
Phenol Red	0.012 g

Final pH: 6.8 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement
Agar, Bacteriological	15 g

Precautions
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 29 g of the medium in 100 mL of purified water until dissolved completely. Filter sterilize.
2. Suspend 15 g of agar in 900 mL of purified water.
3. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
4. Autoclave at 121°C for 15 minutes.
5. Cool sterilized agar to 45 - 50°C and aseptically add the sterile Urea Agar Base.
6. Mix thoroughly and dispense into sterile tubes. Place tubes in a slanted position.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, with soft lumps, and off-white.

Prepared Appearance: Prepared medium is trace to slight hazy, and light to medium yellow-orange.

Expected Cultural Response: Cultural response on Urea Agar Base supplemented with agar, incubated at 35 ± 0.2°C, and examined for growth after 18 - 24 hours.

Microorganism	Approx. Inoculum (CFU)	Expected Results - Urease Reaction
Escherichia coli ATCC® 25922	Direct Inoculation	Negative
Klebsiella pneumoniae ATCC® 13883	Direct Inoculation	Weak positive
Proteus vulgaris ATCC® 13315	Direct Inoculation	Positive
Salmonella typhimurium ATCC® 14028	Direct inoculation	Negative

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Use a heavy inoculum from a pure 18 – 24 hour culture. Inoculate by streaking back and forth over the entire slant surface. Do not stab the butt because it serves as a color control.
2. Incubate tubes with loose caps at 35 ± 2°C.
3. Observe reactions after 6 and 24 hours, and for the next 6 days.[1] Longer periods of incubation may be necessary.

Results

The production of urease is a positive reaction, indicated by an intense red or pink color on the slant.

No color change of the medium is a negative reaction.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 8°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones or other proteins that raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea. Do not autoclave medium because excessive heat may alter ingredients.[8]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. Urea Agar Base detects rapid urease activity of only the urease-positive *Proteus* spp.

Packaging

Urea Agar Base	**Code No.**	**7226A**	**500 g**
		7226B	**2 kg**
		7226C	**10 kg**
Supplement			
Agar Bacteriological	**Code No.**	**7178A**	**500 g**

References

1. **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.
2. **Ewing, W. H.** 1946. An additional *Shigella paradysenteriae* serotype. J. Bacteriol. **51:**433-445.
3. **Ewing, W. H., and D. W. Bruner.** 1947. Selection of *Salmonella* and *Shigella* cultures for serological classification. Am. J. Clin. Pathol. **17:**1-12.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** FDA Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
8. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.

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