

LYSINE IRON AGAR (7211)

Intended Use

Lysine Iron Agar is used for the differentiation of microorganisms on the basis of lysine decarboxylase and hydrogen sulfide production.

Product Summary and Explanation

Lysine Iron Agar is prepared according to the formulation of Edwards and Fife, who developed the medium to detect *Salmonella arizonae*.¹ *S. arizonae* ferments lactose rapidly, and the authors found expected H₂S production on Triple Sugar Iron Agar was suppressed. Detection of *S. arizonae* is important because it has been implicated in food borne infections. By eliminating lactose and incorporating lysine, Edwards and Fife devised a medium differentiating enteric bacilli based on their ability to decarboxylate or deaminate lysine and produce abundant hydrogen sulfide. This medium is recommended for detecting rapid lactose-fermenting *S. arizonae*.

Lysine Iron Agar is specified in standard methods for *Salmonella* testing.²⁻⁶

Principles of the Procedure

Enzymatic Digest of Gelatin provides carbon, nitrogen, and amino acids required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth, and additional sources of nitrogen and carbon. Dextrose is an energy source. L-Lysine is the substrate used to detect lysine decarboxylase and lysine deaminase enzymes. Ferric Ammonium Citrate is an indicator of hydrogen sulfide production. Sodium Thiosulfate is added as a source of inorganic sulfur. Bromcresol Purple, a pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Gelatin	5 g
Yeast Extract.....	3 g
Dextrose.....	1 g
L-Lysine.....	10 g
Ferric Ammonium Citrate.....	0.5 g
Sodium Thiosulfate	0.04 g
Bromcresol Purple	0.02 g
Agar	*13.5 g

*10 -15 g according to gel strength

Final pH: 6.7 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.

Directions

1. Suspend 33 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Dispense into test tubes and autoclave at 121°C for 15 minutes.
4. After autoclaving, allow medium to solidify in a slanted position.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and gray to grayish beige.

Prepared Appearance: Prepared medium is red-purple and trace to slightly hazy.

Expected Cultural Response: Cultural response on Lysine Iron Agar at 35 ± 2°C after 18 – 48 hours incubation.

Microorganism	Approx Inoculum (CFU)	Response	Reactions		
			Slant	Butt	H ₂ S
<i>Citrobacter freundii</i> ATCC® 8090	Direct Inoculation	Growth	K	A	+
<i>Escherichia coli</i> ATCC® 25922	Direct Inoculation	Growth	K	K	---
<i>Proteus mirabilis</i> ATCC® 12453	Direct Inoculation	Growth	R	A	---
<i>Salmonella typhimurium</i> ATCC® 14028	Direct Inoculation	Growth	K	K	+

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

KEY: K, alkaline, R, red (oxidative deamination), A, acid +, H₂S produced, ---, H₂S not produced

Test Procedure

1. Inoculate medium by stabbing base of tube butt and streaking slant with a needle.
2. Loosely cap the tube to ensure aerobic conditions. Incubate at 35°C for 18 - 48 hours.
3. Examine at 18 – 24 and 40 – 48 hours for growth and color changes in tube butt and slant, and for blackening at the apex of slant.

Results

- A positive lysine decarboxylase reaction is purple (alkaline) butt, purple slant. A negative reaction is yellow (acid) butt, purple (alkaline) slant.
- A positive lysine deaminase reaction is a red slant. A negative reaction is a purple slant. (*Proteus* spp. and *Providencia* spp. produce a red slant over a yellow [acid] butt.)
- A positive hydrogen sulfide reaction is blackened medium at the apex of the slant.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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.

Limitations of the Procedure

1. *Salmonella paratyphi* A, unlike other *Salmonella* spp., does not produce lysine decarboxylase resulting in an alkaline slant and an acid butt.
2. H₂S-producing *Proteus* spp. do not blacken the medium.^{2,7} It is suggested that Lysine Iron Agar be used in conjunction with Triple Sugar Iron Agar or other media to confirm differentiation.
3. The reaction of *Morganella morganii* may be variable after 23 hours incubation and may require longer incubation.⁷

Packaging

Lysine Iron Agar	Code No.	7211A	500 g
		7211B	2 kg
		7211C	10 kg

References

1. **Edwards, P. R., and M. A. Fife.** 1961. Lysine-iron agar in the detection of *Arizona* cultures. *Appl. Microbiol.* **9**:478.
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3. **Vanderzant, C. and D. F. Splittstoesser (eds.)**. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1992. Pathogens in milk and milk products, p. 103-212. In R. T. Marshall, (eds.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

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6. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
7. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. *In* Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, M.D.
8. **Finegold, S. M., and W. J. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed. The CV Mosby Company, St. Louis, MO.

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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