



LYSOSTAPHIN TEST KIT

INTENDED USE

Remel Lysostaphin Test Kit is recommended for use in qualitative procedures to differentiate between *Staphylococcus* spp. and *Micrococcus* spp.

SUMMARY AND EXPLANATION

In 1964, Schindler and Schuhardt isolated lysostaphin, an extracellular enzyme, from *Staphylococcus staphylolyticus*.¹ Lysostaphin was found to lyse the cell wall of other species of *Staphylococcus* but not *Micrococcus* spp. Klesius and Schuhardt further demonstrated the use of lysostaphin in the isolation of polymerized DNA.² Schleifer and Kloos used an agar overlay technique to demonstrate lysostaphin activity and differentiate *Staphylococcus* spp. from *Micrococcus* spp.³ In further testing, Geary and Stevens evaluated a rapid lysostaphin test which reliably differentiated staphylococci (sensitive) from micrococci (resistant) in 2 hours.⁴

PRINCIPLE

Lysostaphin is an endopeptidase which cleaves the interpeptidic pentaglycin bridges in the cell wall of staphylococcal peptidoglycan. The sensitivity of various strains of staphylococci is dependent on the contents of the interpeptidic bridge. *Staphylococcus saprophyticus*, *Staphylococcus haemolyticus*, and *Staphylococcus hominis* have been found to be less susceptible than *Staphylococcus aureus* to lysostaphin due to the serine contained in their interpeptide bridge. These cross bridges are found in all staphylococci but not in micrococci.

REAGENTS (CLASSICAL FORMULA)*

Sodium Chloride USP (CAS 7647-14-7).....	8.5 g
Sodium Phosphate Dibasic (CAS 7558-79-4)	1.096 g
Sodium Phosphate Monobasic (CAS 7558-80-7).....	0.315 g
Lysostaphin (CAS 9011-93-2)	0.050 g
Glacial Acetic Acid Buffer (CAS 64-19-7)	100.0 ml
Demineralized Water (CAS 7732-18-5).....	1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE

Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines.^{5,6}

MATERIALS SUPPLIED

(1) Lysostaphin Solution and (2) Phosphate Buffer.

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternate environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Pipettes, (7) Test tubes, (8) Sterile physiological saline (REF R064432).

PROCEDURE

Reconstitute Lysostaphin Solution by adding the entire contents of the Phosphate Buffer vial to the Lysostaphin Solution vial. Mix together well. Store Lysostaphin Solution at 2-8°C for up to 30 days after preparation.

1. Prepare a suspension of the test isolate from a pure, 18-24 hour culture in 0.2 ml of sterile saline. Positive and negative control organisms should be set up concurrently.
2. Add 0.2 ml of Lysostaphin Solution to each test and control organism suspension.
3. Incubate the tubes aerobically at 35-37°C for 2 hours. Do not disturb suspensions before 2 hours.
4. Observe for clearing of the solution.

INTERPRETATION

Positive test - Solution clears, no turbidity remains
Negative test - Solution remains turbid, does not clear

QUALITY CONTROL

All lot numbers of Lysostaphin Test Kit have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
<i>Staphylococcus aureus</i> ATCC® 25923	Aerobic, 2 h @ 35-37°C	Positive
<i>Staphylococcus epidermidis</i> ATCC® 12228	Aerobic, 2 h @ 35-37°C	Positive
<i>Micrococcus luteus</i> ATCC® 4698	Aerobic, 2 h @ 35-37°C	Negative

BIBLIOGRAPHY

1. Schindler, C.A. and V.T. Schuhardt. 1964. Proc. N.A.S. 51:414-421.
2. Klesius, P.H. and V.T. Schuhardt. 1968. J. Bact. 95:739-743.
3. Schleifer, K.H. and W.E. Kloos. 1975. J. Clin. Microbiol. 1:337-338.
4. Geary, C. and M. Stevens. 1986. J. Clin. Microbiol. 23:1044-1045.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
6. Winn, W., S. Allen, J. William, E. Koneman, G. Procop, P. Schreckenberger, and G. Woods. 2006. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams and Wilkins, Baltimore, MD.

PACKAGING

REF R21130, Lysostaphin Test Kit..... 25 Tests/Kit

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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CAS (Chemical Abstracts Service Registry No.)

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