



Instructions for Use

LYSOSTAPHIN DIFFERENTIATION DISKS

[Cat. no. Z112](#)

Lysostaphin Differentiation Disks

50 disks/vial

INTENDED USE

HardyDisk™ Lysostaphin Differentiation Disks are used to rapidly differentiate *Staphylococcus* spp. and *Micrococcus* spp. based on lysostaphin resistance.

SUMMARY

Susceptibility to lysostaphin is a rapid test method in differentiating staphylococci from micrococci. Other differentiation tests, such as glucose fermentation, bacitracin or furazolidone susceptibility tests require at least 24 hours incubation. In contrast, the lysostaphin disk test is a 2.5 hour test.

The peptidoglycan in the cell walls of most *Staphylococcus* contain an interpeptide bridge consisting of glycine-rich peptides. Lysostaphin is an endopeptidase that cleaves these peptide linkages, rendering the cells susceptible to osmotic lysis (lysostaphin susceptible) ^(2,4). The interpeptide bridge of *Micrococcus* does not contain glycine. Since the presence of glycine is essential for the action of lysostaphin, the *Micrococcus* cells are not affected and are lysostaphin resistant. ^(2,4)

FORMULA

Each HardyDisk™ Lysostaphin Differentiation Disk is a filter paper disk (0.25 inch in diameter) containing 20ug of lysostaphin.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. Product should not be used if there are any signs of deterioration, discoloration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

The expiration dating 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 quality control incubation times.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

PROCEDURE

Specimen Collection: This product is not intended for the 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.

1. Allow disks to equilibrate to room temperature before opening vial.
2. Using a pure 18-24 hour culture, prepare a heavy suspension (equivalent in density to a McFarland 2.0 standard) in 2ml of sterile saline.
3. Transfer 1ml of the bacterial suspension into a second test tube.
4. Add one lysostaphin disk to one of the bacterial suspensions and shake vigorously. Use the second suspension as a negative control.
5. Incubate both suspensions aerobically at 35-37°C. for 2.5 hours in an incubator, or for 2 hours in a 35-37°C. waterbath. Do not disturb suspension before 2 hours.
6. After incubation, compare the turbidity of the bacterial suspension with the lysostaphin disk against the control suspension.

INTERPRETATION OF RESULTS

Positive Reaction: Complete clearing of the bacterial suspension or marked decrease in its turbidity as compared with the negative control suspension, indicates susceptibility to lysostaphin (*Staphylococcus* spp.).

Negative Reaction: The suspension remains turbid and does not clear, indicates resistance to lysostaphin (*Micrococcus* spp.).

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Certain *Staphylococcus* species (i.e., *S. aureus*, *S. simulans*, *S. cohnii*, and *S. xylosus*) are more susceptible to lysostaphin than others (e.g., *S. hominis*, *S. saprophyticus* , and *S. haemolyticus*); making standardization and interpretation of this test sometimes difficult. ⁽⁴⁾

Glycine is an important part of the staphylococcal cell wall and is essential for the action of lysostaphin. ^(2,4,5) For optimal results with the lysostaphin test, organisms should be grown on a beef peptone-based medium (which are high in glycine) such as Tryptic Soy Agar, Nutrient Agar or Chocolate Agar. ^(4,5)

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, culture media, test tubes, McFarland 2.0 standard, 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Staphylococcus aureus</i> ATCC ® 25923	D	2.5hr	35°C	Aerobic	Positive (lysostaphin susceptible); complete clearing of bacterial suspension or marked decrease in its turbidity as compared with the negative control
<i>Staphylococcus epidermidis</i> ATCC ® 12228	D	2.5hr	35°C	Aerobic	Positive (lysostaphin susceptible); complete clearing of bacterial suspension

					or marked decrease in its turbidity as compared with the negative control
<i>Micrococcus luteus</i> ATCC® 49732	D	2.5hr	35°C	Aerobic	Negative (lysostaphin resistant); bacterial suspension remains turbid

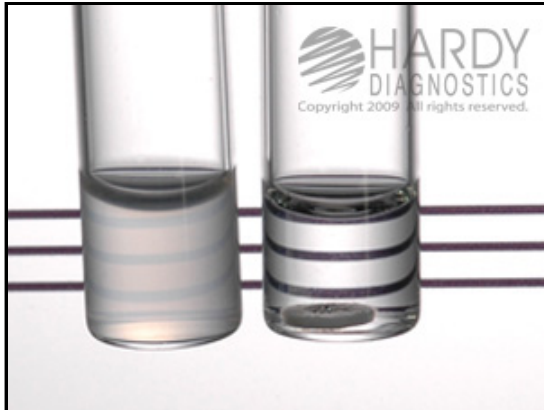
* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

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 certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

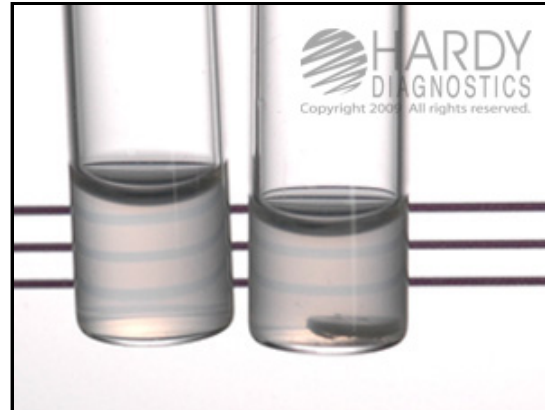
PHYSICAL APPEARANCE

HardyDisk™ Lysostaphin Differentiation Disks are 0.25 inch (in diameter) filter paper disks with the letters LYS ID printed on both sides, and should appear white in color.



Showing positive reaction (lysostaphin susceptible).

A 1mL aliquot of a heavy *Staphylococcus aureus* (ATCC® 25923) suspension (McFarland 2.0) in saline was incubated aerobically for 2.5 hours at 35°C. with a Lysostaphin Differentiation Disk (Cat. no. Z112). The loss of turbidity was indicative of a positive reaction (lysostaphin susceptible), as compared to the negative control (LEFT).



Showing negative reaction (lysostaphin resistant).

A 1mL aliquot of a heavy *Micrococcus luteus* (ATCC® 49732) suspension (McFarland 2.0) in saline was incubated aerobically for 2.5 hours at 35°C. with a Lysostaphin Differentiation Disk (Cat. no. Z112). No loss of turbidity was indicative of a negative reaction (lysostaphin resistant) as compared to the negative control (LEFT).

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.
2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
5. Gunn, B.A. 1981. *Journal of Clinical Microbiology*, Vol.14; 195-200, no.2.
6. Baker, John S. 1984. *Journal of Clinical Microbiology*, Vol.19; 875-879, no.6.

ATCC is a registered trademark of the American Type Culture Collection.

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1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: www.HardyDiagnostics.com

Email: TechService@HardyDiagnostics.com

[Ordering Information](#)

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