

remel

POLYSORBATE 80 HYDROLYSIS SUBSTRATE CONCENTRATE

INTENDED USE

Remel Polysorbate 80 Hydrolysis Substrate Concentrate is recommended for use in qualitative procedures to aid in the differentiation of *Mycobacterium* spp., particularly the scotochromogens and nonphotochromogens.

SUMMARY AND EXPLANATION

Nonpathogenic slow-growing scotochromogens and nonphotochromogens commonly produce lipase which hydrolyzes polysorbate 80 into oleic acid and polyoxyethylated sorbitol, whereas pathogenic *Mycobacterium* spp. do not.¹ The test for detecting polysorbate 80 hydrolysis was introduced by Wayne, in 1962.² Kilburn et al. developed a more stable polysorbate hydrolysis test in 1973.^{3,4}

PRINCIPLE

Some strains of *Mycobacterium* produce an enzyme which splits polysorbate 80 into free oleic acid and polyoxyethylated sorbitol. Polysorbate 80 Hydrolysis Substrate Concentrate contains neutral red dye which is an indicator. Neutral red dye is normally red at pH 7.0, but when bound by polysorbate 80 it takes on the amber or straw color that it has at an alkaline pH. In the presence of certain *Mycobacterium* spp., polysorbate 80 is hydrolyzed and no longer available to bind the indicator causing the neutral red dye to revert to a red color.^{5,6}

REAGENTS (CLASSICAL FORMULA):*

Neutral Red (CAS 553-24-2).....	1.0 g
Disodium Phosphate Buffer (CAS 7558-79-4).....	611.0 ml
Monopotassium Phosphate Buffer (CAS 7778-77-0).....	389.0 ml
Polysorbate 80 (CAS 9005-65-6).....	250.0 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

This product is ready for use and no further preparation is necessary. 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. Protect product from light.

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, TRANSPORT

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

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Sterile demineralized water, (7) Pipettes, (8) Sterile test tubes.

PROCEDURE

Follow established laboratory safety procedures when working with acid-fast cultures and specimens. Consult appropriate references when necessary for detailed procedural information on specimen processing and media inoculation.^{5,6}

1. Add 2 drops of Polysorbate 80 Hydrolysis Substrate Concentrate to 1 ml of sterile demineralized water.
2. Inoculate this mixture with 1 loopful of the test isolate from a young, actively-growing culture slant. Inoculate a positive and negative control simultaneously.
3. Incubate aerobically at 35-37°C and examine at 1, 5, and 10 days.
4. Do not shake the tubes while reading them. Record the number of days required for the first appearance of a pink color. Hold 10 days before discarding as negative.

INTERPRETATION

Positive Test - Pink to iodine-red color development in 10 days or less
Negative Test - Amber color remaining after 10 days of incubation

QUALITY CONTROL

All lot numbers of Polysorbate 80 Hydrolysis Substrate Concentrate have been tested using the following quality control organisms and 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>Mycobacterium kansasii</i> ATCC® 12478	Aerobic, 10 days @ 35-37°C	Positive
<i>Mycobacterium intracellulare</i> ATCC® 13950	Aerobic, 10 days @ 35-37°C	Negative

LIMITATIONS

1. Sedimented cells may appear red in the amber-colored fluid. This is a negative test. The entire liquid medium must turn pink to red for the test to be interpreted as positive.⁵
2. If there is bleaching of the supernatant fluid during incubation, repeat the test. If bleaching reoccurs, record the test as negative.⁵




BIBLIOGRAPHY

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4. Kent, P.T. and G.P. Kubica. 1985. Public Health Microbiology A Guide for the Level III Laboratory. U.S. Dept. of H.H.S., CDC, Atlanta, GA.
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.
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PACKAGING

Polysorbate 80 Hydrolysis Substrate Concentrate:
REF R212765 ml/Tube

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.)

IFU 21276, Revised December 17, 2008

Printed in U.S.A.

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