
remel

PYR REAGENT

INTENDED USE

Remel PYR Reagent is recommended for use in qualitative procedures for rapid presumptive identification of group A streptococci and enterococci.

SUMMARY AND EXPLANATION

In 1981, Godsey, Schulman, and Eriquez described a test to differentiate group A streptococci and enterococci from other streptococci based on the ability to cleave L-pyrrolidonyl- β -naphthylamide (PYR).¹ In 1982, Facklam, Thacker, Fox, and Eriquez used the PYR test in conjunction with the CAMP and bile esculin tests to presumptively identify streptococci.² Facklam et al. incorporated PYR substrate into agar and tested for enzyme activity after overnight incubation. Bosely et al. incorporated PYR substrate into a broth and tested for enzyme hydrolysis after 4 hours incubation.³ Ellner et al. described a colorimetric method for the PYR test using filter paper strips containing PYR substrate.⁴ A positive test was visible within 15-20 minutes. The ease of performance and interpretation of the PYR test, coupled with the evidence cited, suggests that the PYR test is more specific than the routine bacitracin test for group A streptococci and is at least as specific as the routine 6.5% NaCl test for enterococci.⁵

PRINCIPLE

L-pyrrolidonyl- β -naphthylamide (PYR) serves as the substrate for the detection of pyrrolidonyl peptidase. Following hydrolysis of the substrate by the peptidase enzyme, the resulting beta naphthylamine produces a red color upon addition of N,N-dimethylaminocinnamaldehyde (PYR Reagent).

REAGENTS (CLASSICAL FORMULA)*

Sodium Lauryl Sulfate (CAS 151-21-3).....	25.0 g
N,N-dimethylaminocinnamaldehyde (CAS 6203-18-5).....	0.15 g
2-Methoxyethanol (CAS 109-86-4)	50.0 ml
Glacial Acetic Acid (CAS 64-19-7).....	25.0 ml
Formamide (CAS 75-12-7).....	20.0 ml
Deminerlized Water (CAS 7732-18-5).....	905.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

CAUTION! May cause irritation to skin, eyes, and respiratory tract. Avoid breathing vapor and eye/skin contact.

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. Refer to Material Safety Data Sheet for additional information.

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. Protect product from exposure to 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.

Note: Precipitate formation or color variation from yellow to orange-red in PYR Reagent is common and does not affect test performance.

SPECIMEN COLLECTION, STORAGE, TRANSPORT

Specimens should be collected and handled following recommended guidelines.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) PYR Broth (REF R062084), PYR/Esculin Disk (REF R21138), or Strep ID Triplate (REF R02382), (7) Applicator sticks.

PROCEDURE**PYR Broth:**

1. Inoculate PYR Broth using 3-5 colonies from a pure, 18-24 hour culture.
2. Incubate the tube aerobically at 35-37°C for 4 hours.
3. After incubation, add 1-2 drops of PYR Reagent to the tube.
4. Observe for a red color development within 1-2 minutes.

PYR/Esculin Disk:

1. Place the disk on the agar surface of an 18-24 hour, pure culture of the isolate to be tested.
2. Inoculate a small area of the disk surface using a sterile loop or wooden applicator stick.
3. Replace the lid and incubate aerobically at 35-37°C for 15 minutes.
4. After interpreting the esculin reaction, dispense 1-2 drops of PYR Reagent onto the disk.
5. Observe for development of a red color within 1-2 minutes.

Strep ID Triplate:

1. Select 3-4 well-isolated colonies from a pure culture of streptococci and streak the colonies across the surface of Section I.
2. Incubate aerobically at 35-37°C for 18-24 hours.
3. Following incubation, add 1-2 drops of PYR Reagent to the agar surface of Section I.
4. Observe for development of a red color within 1-2 minutes.

INTERPRETATION

Positive Test - Red color development

Negative Test - Yellow color development or no color change

QUALITY CONTROL

All lot numbers of PYR Reagent have been tested using the following quality control organisms and found to be acceptable. Testing of positive and negative controls 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>Streptococcus pyogenes</i> ATCC® 19615	Aerobic, 24 h @ 35-37°C	Positive
<i>Streptococcus agalactiae</i> ATCC® 12386	Aerobic, 24 h @ 35-37°C	Negative

BIBLIOGRAPHY




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3. Bosely, G.S., R.R. Facklam, and D. Grossman. 1983. J. Clin. Microbiol. 18:1275-1277.
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PACKAGING

REF R21258, PYR Reagent 25 ml/Btl

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)

ATCC® is a registered trademark of American Type Culture Collection. CAS (Chemical Abstracts Service Registry No.)

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