

# Instructions for Use

## PYR TEST KIT AND PYR REAGENT

<a href="#">Cat. no. Z75</a>	PYR Test Kit	25 tests/kit
	Kit contains: Z75D - PYR Test Disks Z75R - Chromogenic Solution (PYR Reagent), 5ml	25 disks/jar 1 bottle
<a href="#">Cat. no. Z175</a>	PYR Test Kit	100 tests/kit
	Kit contains: Z175D - PYR Test Disks Z175R - Chromogenic Solution (PYR Reagent), 20ml	100 disks/jar 1 bottle
<a href="#">Cat. no. Z275</a>	PYR Test Kit	75 tests/kit
	Kit contains: Z275C - PYR Test Cards Z275R - Chromogenic Solution (PYR Reagent), 15ml	25 cards/jar 1 bottle
* <a href="#">Cat. no. Z127</a>	PYR Reagent	15ml
* This product sold separately		

## INTENDED USE

Hardy Diagnostics PYR Test Kit and PYR Reagent are recommended for the detection of pyrolydonyl arylamidase (also called pyrolydonyl aminopeptidase) activity in certain groups of bacteria, such as *Streptococcus pyogenes* (group A strep), *Enterococcus* spp., some coagulase-negative staphylococci, and some *Enterobacteriaceae*.

## SUMMARY

PYR is a rapid colorimetric method for presumptive identification of certain groups of bacteria based on the activity of the enzyme pyrolydonyl arylamidase. L-pyroglutamic acid beta-naphthylamide is impregnated into the test disk or card and serves as the substrate for the detection of pyrolydonyl arylamidase. Hydrolysis of the substrate yields beta-naphthylamide which combines with the PYR Reagent (p-dimethylamino-cinnamaldehyde) to form a bright pink to cherry red color. A positive PYR tests allows for the presumptive identification of group A streptococci ( *Streptococcus pyogenes* ) and group D enterococci. In addition, investigators have determined that PYR activity is a key test for differentiation of some species of coagulase-negative *Staphylococcus* and for some genera of the family *Enterobacteriaceae* .

## REAGENT FORMULA

The PYR Test Kit contains disks (Cat. nos. Z75 and Z175) or filter paper (Cat. no. Z275) impregnated with L-pyroglutamic acid beta-naphthylamide and a reagent bottle of chromogenic solution (PYR Reagent); 0.015% p-

dimethylaminocinnamaldehyde.

## STORAGE AND SHELF LIFE

Upon receipt store at 2-8°C. Do not remove PYR test cards (Cat. no. Z275C) from the sealed bag until ready to use. Products should not be used if there are any signs of deterioration or if the expiration date has passed. Protect reagents from freezing.

The expiration date 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 incubation times as stated below.

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 Precautions for blood. 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." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

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

## PROCEDURE

**Specimen Collection:** This product is not intended for 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.

**Method of Use:** The Chromogenic Solution (PYR Reagent) is designed for use in the PYR Test Kit or with the StrepQuick™ (Cat. no. Z122). Moisten the PYR Disk or filter paper slightly with distilled or deionized water. **Do not saturate.** Alternatively, the disk may be placed on a blood agar plate, which will adequately moisten the disk. Using a sterile loop, pick 2-3 well isolated, 18-24 hour colonies (grown on a non-selective media such as Blood Agar, Cat. no. A10) and rub into a small area of the PYR Disk or filter paper so that there is a visible paste. After the test organism has been inoculated onto the disk or filter paper, allow it to react for two minutes. After this incubation period add one drop of Chromogenic Solution (PYR Reagent).

**Note:** Refer to the Instructions for Use (IFU) for [StrepQuick™](#) for procedural use and interpretation of results.

## INTERPRETATION OF RESULTS

A bright pink or cherry red color will appear within one minute if the test is positive. A negative test is indicated by no color change. The development of an orange, salmon, or yellow color should be interpreted as a negative reaction.

**Organisms expected to give a positive result:**

- Group A streptococci (*Streptococcus pyogenes*)
- Group D enterococci (*Enterococcus* spp.)
- Coagulase-negative *Staphylococcus* spp.: *haemolyticus*, *lugdunensis* and *schleiferi*
- *Citrobacter*, *Klebsiella*, *Yersinia*, *Enterobacter* and *Serratia* spp.

## LIMITATIONS

Removing PYR test cards (Cat. no. Z275C) from the sealed bag during long-term storage may result in a decreased shelf life. Maintain cards in the sealed bag until ready to use for best performance.

PYR may be used in the presumptive separation of group A streptococci and group D enterococci from other streptococci. Additional testing, using a pure culture, is recommended for complete identification.

It is important that testing first be performed to determine that the organism is in the *Streptococcus* genus. Only group A streptococci and group D enterococci are PYR-positive. Other streptococci are negative; however additional testing, using a pure culture, may be necessary to separate group A streptococci (*S. pyogenes*) from beta-hemolytic enterococci.

A Gram stain and catalase test should be performed to confirm the presence of gram-positive, catalase-negative cocci. The ability to hydrolyze bile esculin may be used to presumptively identify group D enterococci.

Some *Staphylococcus*, *Aerococcus*, *Lactococcus*, most *Corynebacterium* (*Arcanobacterium*) *haemolyticum*, as well as some *Enterobacteriaceae* and other gram-negative bacilli, are also PYR-positive.

Rare gram-positive cocci will be positive in this test but are not enterococci or group A streptococci. These organisms appear in a Gram stained smear arranged in tetrads or clusters. Additionally, the organisms produce tiny colonies and are not significant pathogens.

A false-negative test can result if the disk or filter paper are too moist.

Weak, pale results occur with the disk test for *Staphylococcus aureus*; positive results may need to be confirmed with other tests or with the tube PYR test, which is available in commercial rapid identification kits.

False-negative tests can result if selective media or tube biochemical agars are used to provide inocula.

Most human infections associated with group D streptococci are caused by *Enterococcus faecalis* and *Streptococcus bovis*.

*Escherichia coli* and indole-positive *Proteus* spp. obtained from media containing a high tryptophan content may yield a blue-green color development. This is a negative result.

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, forceps, StrepQuick™ (Cat. no. Z122), Blood Agar (Cat. no. A10), 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Reaction
<i>Enterococcus faecalis</i>	

ATCC® 29212	Positive; pink to cherry red color change
<i>Enterobacter aerogenes</i> ATCC® 13048	Positive; pink to cherry red color change
<i>Streptococcus agalactiae</i> ATCC® 12386	Negative; no color change
<i>Escherichia coli</i> ATCC® 25922	Negative; blue color change

## QUALITY CONTROL

### USER QUALITY CONTROL

It is recommended that each new lot or shipment be tested with known positive and negative controls.<sup>(1,5)</sup>

### PHYSICAL APPEARANCE

- PYR Test Disks and filter paper should appear white in color. Disks and filter paper should not be used if they appear pink or red in color.
- Chromogenic Solution (PYR Reagent) should appear clear and colorless with no precipitate.



**Showing positive PYR reaction with the PYR Test Kit (Cat. no. Z75).**

*Enterococcus faecalis* (ATCC® 29212) growth was applied to a PYR Disk moistened with deionized water and incubated at room temperature for two minutes. Subsequently, one drop of Chromogenic Solution (PYR Reagent) was applied to the disk. The pink to red color development was indicative of a positive PYR reaction.



**Showing negative PYR reaction with the PYR Test Kit (Cat. no. Z75).**

*Streptococcus agalactiae* (ATCC® 12386) growth was applied to a PYR Disk moistened with deionized water and incubated at room temperature for two minutes. Subsequently, one drop of Chromogenic Solution (PYR Reagent) was applied to the disk. No pink to red color development was indicative of a negative PYR reaction.



PYR Test Kit (Cat. no. Z75).

## 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. Versalovic, J., 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. Ellner, P.D., et al. 1985. Preliminary evaluation of a rapid colorimetric method for the presumptive identification of group A streptococci and enterococci. *J. Clin. Microbiol.*; 22:880-881.
- Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

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[Ordering Information](#)

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