

Instructions for Use

STREPQUICK™

Cat. no. Z122	StrepQuick™	25 tests/kit
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INTENDED USE

Hardy Diagnostics StrepQuick™ test kit is intended to aid in the identification of gram-positive, catalase-negative cocci based on pyroglutamate aminopeptidase (PYR), leucine aminopeptidase (LAP), and esculin hydrolysis (ESC) activity. This test kit simplifies identification of *Enterococcus* spp. and group A streptococci (*Streptococcus pyogenes*).

SUMMARY

Hardy Diagnostics StrepQuick™ is a rapid means of determining three key biochemical reactions for the differentiation and identification of gram-positive, catalase-negative cocci. StrepQuick™ detects activity of three enzymes; pyroglutamate aminopeptidase (PYR), leucine aminopeptidase (LAP), and esculinase (ESC). A positive PYR reaction is characteristic of group A streptococci and *Enterococcus* spp.⁽¹⁾ *Aerococcus viridans* and *Leuconostoc* spp. typically show negative reactions for LAP activity.⁽¹⁾ *Enterococcus* spp. and group D streptococci are positive for esculin hydrolysis.

PYR is a rapid colorimetric method for the detection of pyroglutamate aminopeptidase activity. L-pyroglutamic acid beta-naphthylamide impregnated into the test card serves as the substrate for the detection of pyroglutamate aminopeptidase. Hydrolysis of the substrate yields beta-naphthylamide which combines with the cinnamaldehyde reagent to form a bright pink to cherry red pigment.

L-leucine-beta-naphthylamide is impregnated in the filter paper in the LAP test circle. This substrate is hydrolyzed by the enzyme leucine aminopeptidase. Like the PYR reaction, released beta-naphthylamide combines with the cinnamaldehyde reagent to form a bright pink to cherry red pigment.

Esculin and ferric ammonium citrate are contained within the third reaction circle. Esculin is hydrolyzed by organisms capable of producing the enzyme esculinase. Esculetin, the end product of the hydrolysis reaction, complexes with ferric ions to produce a light gray to gray color.

FORMULA

Pyroglutamic aminopeptidase (PYR):

L-pyroglutamic acid beta-naphthylamide

Leucine aminopeptidase (LAP):

L-leucine-beta-naphthylamide

Esculin hydrolysis (ESC):

Esculin

Ferric Ammonium Citrate

StrepQuick™ Developer:

p-dimethylaminocinnamaldehyde

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. The test kit should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and 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 or serological tests to identify cultures of isolated organism.

Method of Use:

Note: Perform Gram stain and catalase test prior to inoculating StrepQuick™. Test organisms should be gram-positive cocci that are aerobic or facultatively anaerobic and catalase-negative.

- 1) Moisten each test circle slightly with a single drop of distilled or deionized water. **Do not saturate.**
- 2) Using a sterile plastic loop, pick 2-3 well isolated, 18-24 hour colonies and rub into a small area of the PYR reaction circle so that there is a visible paste.
- 3) Repeat step 2 for the LAP and ESC test circles.

Note: Take care not to carry over substrate from one circle to the next.

- 4) After the test organism has been inoculated onto the test circles, allow it to react for ten minutes.
- 5) After the 10 minute incubation period add one drop of chromogenic developer to the PYR and LAP circles.
- 6) Immediately after addition of the chromogenic developer, observe for the development of a bright pink or cherry red color in the PYR and LAP circles. The reaction should be read only in the area of the card where the organism was inoculated. A diffuse, light pink color covering the entire test area is not considered positive and should be disregarded. Any color development in the PYR and LAP circles appearing after 1 minute should be disregarded. Color development of orange, yellow or salmon in the PYR circle should be interpreted as a negative reaction.
- 7) Observe for a light gray to gray color to form in the ESC circle. Any darkening at all should be considered positive. Allow 10-15 minutes for full color development.

INTERPRETATION OF RESULTS

Test	Positive Reaction	Negative Reaction
PYR	Cherry red or bright pink color develops	Development of a orange, yellow or salmon color or no change
LAP	Cherry red or bright pink color develops	No color development*
ESC	Light gray to gray color develops	Any color other than gray

* Refer to Procedure step 6 above.

Expected reactions of select organisms:^(1,5-7)

Organism	Expected Reactions						
	PYR	LAP	ESC	Vancomycin (30ug)**	6.5% NaCl*	Hemolysis*	Gram Morphology*
<i>Aerococcus viridans</i>	+	-	V	S	+	Alpha	Cl
<i>Aerococcus urinae</i>	-	+	V	S	+	Alpha	Cl
<i>Enterococcus</i> spp.	+	+	+	S/R	+	Alpha/Gamma	Ch
<i>Gemella haemolysans</i>	+	+	-	S	-	Alpha/Gamma	D,Cl,Ch,T
<i>Gemella morbillorum</i>	+	+	-	S	-	Alpha/Gamma	D,Cl,Ch,T
<i>Leuconostoc</i> spp.***	-	-	V(+)	R	V(+)	Gamma	Ch
<i>Pediococcus</i> spp.	-	+	+	R	V(-)	Alpha	Cl,T
<i>Streptococcus pyogenes</i> (group A)	+	+	V(-)	S	+	Beta	Ch
<i>Streptococcus agalactiae</i> (group B)	-	+	-	S	V(+)	Beta	Ch
<i>Streptococcus</i> group D (non- <i>Enterococci</i>)	-	+	+	S	-	Alpha/Beta/Gamma	Ch
<i>Streptococcus pneumoniae</i>	-	+	V(-)	S	-	Alpha	D
<i>Streptococcus viridans</i>	-	+	V(-)	S	-	Alpha/Gamma	Ch

V = Variable results

V(+) = Variable reactions where the majority of isolates are positive (>80%)

V(-) = Variable reactions where the majority of isolates are negative (>80%)⁽⁹⁾

S = Sensitive

R = Resistant

Cl = Cluster

Ch = Chain

D = Diplococci

T = Tetrad

* These tests are not included in the StrepQuick™ card, but are included to aid in identification.

** Results are included to help aid in identification, as this test separates *Leuconostoc* and *Pediococcus* spp. from the majority of listed species.

*** *Weissella paramesenteroides* ATCC® 33313 (previously known as *Leuconostoc paramesenteroides*) is consistently positive for LAP.⁽¹⁰⁾

LIMITATIONS

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

A Gram stain and catalase test should be performed to confirm the presence of gram-positive, catalase-negative cocci.

Only pure cultures or isolated colonies of gram-positive, catalase-negative cocci should be tested; mixed cultures may give false-positive reactions.

Staphylococcus, *Aerococcus*, most *Corynebacterium haemolyticum*, as well as some Enterobacteriaceae and other gram-negative bacilli, are PYR positive.^(1,9)

For PYR and LAP tests, after addition of the developer, color development after 1 minute should be disregarded.⁽⁵⁾

For PYR and LAP test, after the addition of the developer, the development of a diffuse, light pink color covering the entire test area is not considered positive and should be disregarded.

False-negative results can occur for any of the reactions if inadequate inoculum is used.⁽⁵⁾

Bile tolerance is not included in the esculin hydrolysis test, so results obtained with StrepQuick™ may not correlate with results obtained from Bile Esculin media.

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, other culture media, swabs, 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 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	PYR Reaction	LAP Reaction	ESC Reaction
<i>Streptococcus agalactiae</i> ATCC® 12386	Negative; no color change	Positive; cherry red or bright pink color change	Negative; no color change
<i>Enterococcus faecalis</i> ATCC® 29212	Positive; cherry red or bright pink color change	Positive; cherry red or bright pink color change	Positive; light gray to gray color change
<i>Aerococcus viridans</i> ATCC® 11563	Positive; cherry red or bright pink color change	Negative; no color change directly on or in the adjacent area of the sample inoculum	Negative; no color change

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 certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.



Showing StrepQuick™ (Cat. no. Z122) reactions for *Enterococcus faecalis* (ATCC® 29212). Growth from 18-24 hour colonies were rubbed onto each reaction circle leaving a visible paste. After a 10 minute room temperature incubation, one drop of chromogenic developer was added to each circle. Showing: PYR Positive (cherry red or bright pink color change); LAP Positive (cherry red or bright pink color change); ESC Positive (light gray to gray color change).

REFERENCES

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