IFU

Instructions for Use

E. COLIPRO™ O157 KIT

Cat. no. PL070HD	E. coliPRO™ O157 Kit	50 tests/kit
	Each kit contains:	
	E. coliPRO™ O157 Latex Reagent	1 dropper vial/kit
	E. coliPRO™ O157 Positive Control Suspension	1 dropper vial/kit
	E. coliPRO™ O157 Negative Control Latex Reagent	1 dropper vial/kit
	Latex Test Cards	20 cards/kit
	Mixing Sticks	75 sticks/kit

INTENDED USE

The Hardy Diagnostics E. coliPRO™ O157 Kit provides a rapid latex agglutination method for the detection of *E. coli* serogroup O157 antigen from colonies isolated from laboratory culture media.

SUMMARY

Escherichia coli serotype O157:H7 is a verotoxin-producing (VT-producing) pathogen. This serotype has been reported as an etiological agent in sporadic and outbreak cases of hemorrhagic colitis. It is also associated with hemolytic uremic syndrome. Certain *E. coli* serotypes, other than O157:H7, also produce verotoxin. However, the diarrhea caused by these other serotypes is not usually bloody. Additionally, *E. coli* serotype O157:H7 does not ferment sorbitol. Therefore, if MacConkey Agar with Sorbitol is used as a primary screen, the colonies of *E. coli* serotype O157:H7 appear colorless as they are non-sorbitol-fermenting, while colonies of other serotypes are pink indicating sorbitol-fermentation.[1-4]

Hardy Diagnostics E. coliPRO™ O157 Kit contains blue latex particles coated with an antiserum against *E. coli* O157 antigen. When the coated latex particles are mixed with fresh colonies of *E. coli* serotype O157 the bacteria will bind to the antiserum, causing the latex particles to visibly agglutinate, indicative of a positive reaction. Bacteria which do not belong to the O157 serotype will not bind to the antiserum and will not result in agglutination, indicative of a negative reaction.[5,6]

MATERIALS SUPPLIED

E. coliPRO™ O157 Latex Reagent	Blue latex particles coated with purified rabbit IgG which react with *E. coli* serotype O157, 3.1ml. Latex particles are suspended in a buffer containing 0.0098% sodium azide as a preservative.
E. coliPRO™ O157 Positive Control Suspension	*E. coli* serotype O157:H7 colonies grown on agar medium, harvested and inactivated, 1.5ml. The antigen is suspended in a buffer containing 0.0095% sodium azide as a preservative.

E. coliPRO™ O157 Negative Control Latex Reagent	Blue latex particles coated with purified rabbit IgG which does not react with *E. coli* serotype O157, 1.5ml. Latex particles are suspended in a buffer containing 0.0098% sodium azide as a preservative.
Latex Test Cards	Disposable white cards with 10 reaction circles
Mixing Sticks	Disposable mixing sticks

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, pasteur pipettes, Saline (Cat. no. K59), timer, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C away from direct light. **Do not freeze**. This kit, or any of its reagents, should not be used if there are any signs of discoloration, contamination, or if the expiration date has passed.

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.

The Latex Reagents contain sodium azide as a preservative. Sodium azide can react explosively with copper or lead if allowed to accumulate. Although the amount of sodium azide in the reagents is minimal, large quantities of water should be used when flushing these reagents down the sink.

Do not use the Latex Reagents if autoagglutination is visible. Autoagglutination is defined as agglutination of E. coliPRO™ O157 Latex Reagent in the absence of added test specimen or agglutination of the Negative Control Latex Reagent with the test specimen or Positive Control Suspension. Autoagglutination may indicate that contamination or reagent deterioration has occurred.

PROCEDURE

Specimen Collection:

For specific procedures regarding specimen collection and preparation of primary cultures consult standard microbiological references.[1-4]

In general, the Latex Test should be performed on isolated non-sorbitol-fermenting colonies that are 18 to 24 hours old. Isolates can be taken from MacConkey Agar with Sorbitol (Cat. no. G36) or CT-SMAC Agar plates (Cat. no. G129).

Test Protocol:

1. Allow all reagents to come to room temperature for at least 10 minutes prior to use.

2. Place one drop of sterile Saline (Cat. no. K59) within the circle on the test card.

3. Select 1-4 well-isolated colonies from the agar surface.

4. Create an emulsion of the colonies by mixing the Saline on the test card.

5. Mix the Latex Reagents, by inverting the tubes, prior to use. Dispense 1 drop of E. coliPRO™ O157 Latex Reagent onto a test circle on the test card. *Important:* Do not allow the Latex Reagent bottle to come into contact with the organism suspension.

6. Mix the Latex Reagent and the organism suspension with the mixing sticks provided, using the complete area of the circle. A new stick should be used for each reagent.

7. Gently hand-rock the entire card, allowing the mixture to flow slowly over the ring area.

8. For up to 2 minutes, under normal lighting conditions, observe for agglutination (strong clumping) of the latex particles.

9. All organisms yielding a positive agglutination reaction should be retested with the Negative Control Latex Reagent. Repeat steps 4-8 with the Negative Control Latex Reagent.

See "Interpretation of Results" and "Limitations" sections for more information.

INTERPRETATION OF RESULTS

Positive Results:

A rapid and significantly strong clumping of the latex particles in under 2 minutes requires additional testing with the Negative Control Reagent to confirm that the observed agglutination reaction was specific for the *E. coli* O157 serotype.

If the organism agglutinates with the Latex Test Reagent and fails to agglutinate with the Negative Control Latex Reagent, this indicates the identification of *E. coli* serotype O157.

If the organism agglutinates with the Latex Test Reagent and also agglutinates with the Negative Control Latex Reagent, this is considered a false-positive reaction due to autoagglutination or cross reaction of the strain.

Negative Results:

If no visible agglutination of the latex particles is observed in any of the reaction circles, there is no need to continue testing with the Negative Control Latex Reagent.

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.

The kit is intended for use in the identification of pure cultures from MacConkey Agar based media, which show

typical *E. coli* morphology.

False-negative or false-positive results can occur if inadequate amounts of culture or Latex Test Reagents are used.

Positive test results should be biochemically confirmed. Conventional serological testing with *E. coli* H antisera should be used to confirm the serotype of a latex agglutination positive culture. Occasionally non-motile *E. coli* O157 are encountered.

E. coliPRO™ O157 Reagents were developed to detect the presence of *E. coli* serogroup O157 antigen. Most non-sorbitol-fermenting colonies that give a positive result in this kit are presumptively identified as *E. coli* O157:H7. Some other *E. coli* O157 strains, for instance *E. coli* O157:H16, that are non-sorbitol-fermenting may also produce a positive reaction in this test.

If stringy or mucoid agglutination reactions are observed between the E. coliPRO™ O157 Latex Reagent and the organism suspension, the reactions are considered uninterpretable and retesting of the organism should occur. It is recommended that a fresh organism suspension be prepared and the colony clumps be allowed to settle out of solution. Only the supernatant should be used in retesting.

Although this test has been specifically developed to reduce the normal cross-reactivity of *Escherichia hermanii*, rare and uncommon strains may cross react. Cellobiose growth in the presence of KCN and yellow pigmentation may be used for differentiation.

Refer to the document "Limitations of Procedures and Warranty" for more information.

QUALITY CONTROL

The following organisms are routinely tested on each lot of the E. coliPRO™ O157 Kit:

Test Organisms	Inoculation Method*	Testing Time	Results
Escherichia coli O157:H7 ATCC® 700728	*	up to 2 min.	Agglutination observed only with the Latex Reagent
Escherichia coli ATCC® 25922	*	up to 2 min.	No agglutination observed only with the Latex Reagent

* 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 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.

END USER QUALITY CONTROL

It is recommended that the performance of the Latex Reagent and the Negative Control Latex Reagent be evaluated with the Positive Control Suspension with each new lot number and new shipment. In accordance with the laboratory's quality control program and/or regulatory guidelines, further periodic testing may be required.

The Latex Reagent should show obvious agglutination with the Positive Control Suspension. The Positive Control Suspension contains *E. coli* O157:H7 antigens from *E. coli* serotype O157:H7 grown on agar medium, harvested, and

inactivated to produce the positive control antigen. If agglutination is observed with the Positive Control Suspension, it is necessary to test the Negative Control Latex Reagent with the Positive Control Suspension to yield no agglutination.

The use of the kit should be continued only if the Latex Reagent agglutinates with the Positive Control Suspension and the Negative Control Latex Reagent fails to agglutinate with the Positive Control Suspension.

If the Latex Reagent fails to agglutinate with the Positive Control Suspension, this indicates the potency of the reagents is low and they need to be discarded.

If the Latex Reagent agglutinates with the Positive Control Suspension and the Negative Control Latex Reagent also agglutinates with the Positive Control Suspension, this indicates the reagents are autoagglutinating and need to be discarded.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

2. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology,* C.V. Mosby Company, St. Louis, MO.

3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook,* Vol. I, II & III. American Society for Microbiology, Washington, D.C.

4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology.* J.B. Lippincott Company, Philadelphia, PA.

5. Broczyk, A., et al. 1987. False-positive identification of *Escherichia coli* in foods. *Int. J. Food Microbiol.* 4:347-349.

6. Thompson, J.S., 1990. Rapid biochemical test to identify verocytotoxin-positive strains of *Escherichia coli* serotype 0157. *J. Clin. Microbiol*. 28:2165-2168.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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