Prolex™ *E. coli* Non-O157 Identification Kit

CE UKCA IVD pro-lab.com/resources 50

REF PL.1070 REF PL.1071 REF PL.1072 REF PL.1073 REF PL.1074 REF PL.1075
REF PL.1076 REF PL.1077

INSTRUCTIONS FOR USE

INTENDED USE

The Prolex™ *E. coli* Non-O157 Identification Kit provides a rapid method to identify six non-O157 *Escherichia coli*: O26, O103, O111, O145, O45 and O121 isolated from cultured specimens.

SUMMARY AND EXPLANATION

Although *E. coli* O157 is the most common cause of STEC illness it has now been recognized that non-O157 strains of *E. coli* cause severe human disease that is comparable with that caused by *E. coli* O157. Of these strains *E. coli* O26 is the most commonly isolated and does cause haemolytic uremic syndrome (HUS) as can several other serotypes [1, 2, 3, 4, 5]. In addition, the following strains are the next most commonly isolated *E. coli* O103, *E. coli* O111, *E. coli* O145, *E. coli* O45 and *E. coli* O121. These serotypes have also been shown to cause significant human disease [6]. In the United States, and the United Kingdom, the CDC (USA) and SERL (UK) have recommended that all clinical laboratories screen for STEC[7, 8].

PRINCIPLE OF THE TEST

Polystyrene beads are sensitized with immunoglobulins against each of the serotype specific *E. coli* somatic antigens (*E. coli* O26, *E. coli* O103, *E. coli* O111, *E. coli* O145, *E. coli* O121 or *E. coli* O45). When the coated polystyrene particles are mixed with fresh organisms of the corresponding *E. coli* serotype the bacteria will bind to the antibody, causing the particles to visibly agglutinate (positive reaction). Bacteria which are not of serotypes O26, O103, O111, O145, O121 or O45 will not bind to the antibody and will not result in agglutination (negative reaction).

MATERIALS PROVIDED

Each Prolex™ *E. coli* Non-O157 Identification Kit (PL.1070) is sufficient for 50 tests. Materials are supplied ready for use.

- Latex Reagents: Six dropper bottles containing 2.5 ml of latex particles coated with purified rabbit IgG that react with *E. coli* somatic antigens (O26, O103, O111, O145, O121 or O45). Polystyrene particles are suspended in a buffer containing 0.094% sodium azide as a preservative. The following six latex reagents are included:
  Prolex™ *E. coli* O26 Latex Reagent (PL.1071)
  Prolex™ *E. coli* O45 Latex Reagent (PL.1072)
  Prolex™ *E. coli* O103 Latex Reagent (PL.1073)
  Prolex™ *E. coli* O111 Latex Reagent (PL.1074)
  Prolex™ *E. coli* O121 Latex Reagent (PL.1075)
  Prolex™ *E. coli* O145 Latex Reagent (PL.1076)
- Prolex™ Negative Control Latex Reagent (PL.1077): One dropper bottle containing 2.5 ml of polystyrene particles coated with purified normal (non-immune) rabbit IgG. Polystyrene particles are suspended in a buffer containing 0.098% sodium azide as a preservative.
- Test cards
- Mixing sticks

MATERIALS REQUIRED BUT NOT PROVIDED

- Phosphate buffered saline (PBS) or Normal saline (0.85% sodium chloride)
- McFarland Standard 3.0, 4.0 or 5.0
- 12 x 75 mm test tubes
- Inoculating loop or needle
- Pasteur pipettes
- Timer

STABILITY AND STORAGE

Reagents should be stored at 2-8°C. **Do not freeze**. Reagents stored under these conditions will be stable until the expiration date shown on the product label.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic use.
2. Do not use the reagents after the expiration date shown on the product label.
3. The reagents contain a small amount of sodium azide. Sodium azide can react explosively with copper or lead plumbing if allowed to accumulate. Although the amount of sodium azide in the reagent is minimal, large quantities of water should be used if reagents are flushed down the sink.
4. Universal precautions should be taken in handling, processing and discarding all clinical specimens. All test materials should be considered potentially infectious during and after use and should be handled and disposed of appropriately.
5. Do not use any of the reagents if autoagglutination is visible. This would appear as agglutination of the test reagent or negative control in the absence of a test isolate.
6. The procedures, storage conditions, precautions, and limitations specified in these directions must be followed to obtain valid test results.
7. Reagents contain materials of animal origin and should be handled as a potential carrier and transmitter of disease.
8. Do not use the kit if the reagent do not yield appropriate results. Refer to “Quality Control.”

PREPARATION OF CULTURES

Clinical specimens should be cultured on media that will facilitate optimal growth, such as MacConkey Agar, Sorbitol MacConkey Agar (SMAC), Chromogenic media, Blood Agar, etc. Suspect colonies may be tested directly or from a subculture. Colonies from an overnight culture (18-24 hours) must be cleanly removed from the agar surface for testing using a sterile loop or needle. Young, fast-growing cultures typically give the best results.

TEST PROCEDURE

1. Allow all reagents to come to room temperature before use.
2. Re-suspend the test latex reagents by gently inverting the dropper bottle several times. Examine the dropper bottles to ensure that the latex particles are properly suspended before use. Do not use if the latex fails to re-suspend.
3. Using a pipette transfer 0.3 ml phosphate buffered saline or normal saline into a 12 x 75 mm culture tube or equivalent.
4. Select sufficient suitable colonies from the test culture with a loop or needle and prepare a suspension in the phosphate buffered saline corresponding to a 3-5 McFarland Standard.
5. Label the test card with each of the serotypes and then add one drop of each latex reagent into the appropriate test circle.
6. Using a pipette transfer one drop (35 µl) of the test suspension onto each of the test circles.
7. Mix each of the test circles with a separate mixing stick.
8. Rock the card gently and examine for agglutination. A positive reaction (agglutination) will be visible within 30 seconds.
9. Isolates that give a positive test with any of the test reagents must be tested further by repeating the procedure using the Prolex™ Negative Control Latex Reagent.

QUALITY CONTROL

The Prolex™ *E. coli* Non-O157 Latex Reagents and the Prolex™ Negative Control Latex Reagent must be tested with phosphate buffered saline or normal saline before running the test isolates. There must be no agglutination in either of the reagents within 30 seconds.

INTERPRETATION OF RESULTS

The following table shows how the results obtained with the Prolex™ *E. coli* Non-O157 Latex Reagents and Prolex™ Negative Control Latex Reagent should be interpreted:

Organisms	Results with Latex Reagents							Interpretation
	***E. coli* O26**	***E. coli* O45**	***E. coli* O103**	***E. coli* O111**	***E. coli* O121**	***E. coli* O145**	**Negative Control**	
E. coli O26	+	-	-	-	-	-	-	Presumptive for *E. coli* O26
E. coli O45	-	+	-	-	-	-	-	Presumptive for *E. coli* O45
E. coli O103	-	-	+	-	-	-	-	Presumptive for *E. coli* O103
E. coli O111	-	-	-	+	-	-	-	Presumptive for *E. coli* O111
E. coli O121	-	-	-	-	+	-	-	Presumptive for *E. coli* O121
E. coli O145	-	-	-	-	-	+	-	Presumptive for *E. coli* O145

Positive Result: Visible agglutination of the latex particles within 30 seconds.

Negative Result: No visible agglutination of the latex particles at 30 seconds.

Uninterpretable Result: If the test isolate agglutinates with both the latex reagent and the Negative Control Latex Reagent, an autoagglutinating or cross-reacting strain is present. Perform further testing to rule out non-O157 *E. coli*. If the test isolate reacts with more than one of the test reagents, the test is uninterpretable.

LIMITATIONS OF THE PROCEDURE

1. Positive test results should be confirmed using routine biochemical testing.
2. Although this test has been developed to reduce cross-reactivity, rare strains can cross-react. Do not observe for agglutination after 30 seconds.
3. If the test isolate fails to react with any one of the test reagents and you suspect that it is a pathogen please send it to your local reference centre for further study.
4. If the test isolate reacts with more than one of the test reagents please send it to your local reference centre for further study.

PERFORMANCE CHARACTERISTICS

The performance of the reagents was evaluated at a PHAC Reference Laboratory. Each of the reagents was tested against 177 different *E. coli* serotypes including STEC.
The results from this evaluation showed 100% specificity and sensitivity for each of the reagents.

An additional study conducted at the Scottish *E. coli* O157/STEC Reference Laboratory confirmed that the Prolex™ *E. coli* Non-O157 Identification Kit demonstrated 100% specificity and sensitivity in detecting the six non-O157 STEC serogroups cultured on Sorbitol MacConkey (SMAC) and Colorex™-STEC media[8].

Cross Reactivity
Each of the reagents was tested against the following enteric bacteria including *Shigella* species for cross reactivity. No cross reactivity were found.

Organism	Result
Aeromonas hydrophila	Negative
Bacillus cereus	Negative
Bacillus subtilis	Negative
Campylobacter coli	Negative
Campylobacter fetus	Negative
Campylobacter jejuni	Negative
Citrobacter braakii (freundii)	Negative
Enterobacter aerogenes	Negative
Enterobacter cloacae	Negative
Enterococcus faecalis	Negative
Escherichia hermanii	Negative
Klebsiella pneumoniae	Negative
Proteus vulgaris	Negative
Pseudomonas aeruginosa	Negative
Salmonella enteriditis	Negative
Salmonella typhimurium	Negative
Serratia marcescens	Negative
Serratia liquefaciens	Negative
Shigella flexneri	Negative
Shigella dysenteriae	Negative
Shigella sonnei	Negative
Staphylococcus aureus	Negative
Vibrio parahaemolyticus	Negative

AVAILABILITY

Cat. No.	Description
PL.1071	Prolex™ *E. coli* O26 Latex Reagent
PL.1072	Prolex™ *E. coli* O45 Latex Reagent
PL.1073	Prolex™ *E. coli* O103 Latex Reagent
PL.1074	Prolex™ *E. coli* O111 Latex Reagent
PL.1075	Prolex™ *E. coli* O121 Latex Reagent
PL.1076	Prolex™ *E. coli* O145 Latex Reagent
PL.1077	Prolex™ Negative Control Latex Reagent
PL.1078	Prolex™ *E. coli* O91 Latex Reagent
PL.091P	Mixing Sticks
PL.092-48	Test cards

REFERENCES

1. **Banatvala N., Debeukelaer M.M., Griffin P.M., et al.** Shiga-like toxin producing *Escherichia coli* O111 and associated haemolytic-uremic syndrome; a family outbreak. Pediatr. Infect. Dis. J. 1996 15:1008-11.
2. **Tarr P.I., Fouser L.S., Stapleton A.E., et al.** Haemolytic-uremic syndrome in a six year girl after a urinary tract infection with Shiga-toxin producing *Escherichia coli* O103:H2. N. Engl. J. Med. 1996 335:635-8.
3. **Brooks J.T., Sowers E.G., Wells J.G., Greene K.D., Griffin P.M., Hoekstra R.M., Strockbine N.A.** Non-O157 Shiga Toxin-Producing *Escherichia coli* Infections in the United States, 1983-2002. J. Infect. Dis. 2005 192:1422-1428.

4. **Beutin L., Zimmermann S., Gleier.** Human Infections with Shiga-Toxin Producing *Escherichia coli* Other Than Serogroup O157 in Germany. Emerging Infectious Disease 1998 4:635-639.
5. **Hadler J.L., Clogher P., Hurd S., Phan Q., Mandour M., Bemis K., Marcus R.** Ten–year trends and risk factors for non-O157 Shiga toxin-producing *Escherichia coli* found through Shiga toxin testing, Connecticut, 2000-2009. Clin. Infect. Dis. 2011 53(3):269-76.
6. **Hermos C.R., Janineh M., Han L.L., McAdam A.J.** Shiga Toxin Producing *Escherichia coli* in Children: Diagnosis and Clinical Manifestations of O157:H7 and Non-O157:H7 Infection. J. Clin. Micro. 2011 49:955-959.
7. **CDC 2009**. Recommendations for diagnosis of Shiga toxin-producing *Escherichia coli* infections in clinical laboratories. MMWR Morb. Mortal. Wkly. Rep 58:1-14.
8. **Mitchel M.C., Davies G., Holmes A., Allison L.** (2025, September 23-25). *Chromogenic agar and agglutination identification of Shiga toxin-producing E. coli* [Poster Presentation]. IBMS Congress, Birmingham, United Kingdom.

SYMBOLS GLOSSARY

Symbol	Meaning
	Manufacturer
	Use-by date
LOT	Lot number
REF	Catalogue number
	Temperature limit
	Consult instructions for use or consult electronic instructions for use
IVD	In vitro diagnostic medical device
	Contains sufficient for <n> tests
CE	Indicates European Conformity
UK CA	UK Conformity Assessed
EC REP	Authorized representative in the European Community / European Union

Pro-Lab Diagnostics U.K.
3 Bassendale Road, Bromborough,
Wirral, Merseyside CH62 3QL
United Kingdom
Tel.: 0151 353 1613
www.pro-lab.co.uk
E-mail: uksupport@pro-lab.co.uk

Pro-Lab Diagnostics Canada
20 Mural Street, Unit #4
Richmond Hill, Ontario L4B 1K3
Canada
Tel.: 905-731-0300
www.pro-lab.com
E-mail: support@pro-lab.com

Pro-Lab Diagnostics U.S.A.
1301 Blue Ridge Drive, Suite #101
Georgetown, Texas 78626-3269
United States
Tel.: 512-832-9145
www.pro-lab-direct.com
E-mail: support@pro-lab.us

EC REP Advena Ltd. Tower Business Centre, 2nd Floor, Tower Street, Swatar, BKR 4013 Malta