

Rapid Test Kit for *E. coli*

Intended Use

Rapid Test Kit for *E. coli* is used for presumptive identification of *E. coli* on the basis of enzyme substrate reaction and Indole test.

Summary

Enzyme substrate tests utilize hydrolysable substrates for detection of *E. coli*. When the enzyme technique is used, *E. coli* are defined as bacteria giving a positive total coliform response in possessing D-galactosidase, which cleaves a chromogenic substrate. Indole test, alone, is not an adequate screen for *E. coli*, since there are at least 52 species of Gram-negative bacilli that grow on MacConkey media, are indole positive and ferment lactose. These include *K. oxytoca*, *Citrobacter freundii*, *Serratia odorifera*, and *Aeromonas hydrophila*. The enzyme-substrate test therefore, in conjunction with Indole test can be used as a rapid screening test to assist in identification of *E. coli*.

Principle

Lactose fermentation is a classical identification test. It is normally demonstrated by acid production after the disaccharide has been cleaved into galactose and glucose by the enzyme-galactosidase. The medium contains all essential nutrients required for growth of *E. coli* along with a synthetic chromogenic substrate Ortho-Nitrophenyl-D-Galactopyranoside (ONPG), which is a substitute for lactose. ONPG is hydrolysed in the same way as lactose, but being chromogenic produces a yellow colour. Using this test, β -galactosidase positive, indole positive, Gram-negative organism can be presumptively identified as *E. coli*. Hydrolysis of ONPG denotes presence of β -galactosidase enzyme and is detected by development of yellow colour in the medium. Indole production is detected by addition of Kovac's Reagent wherein development of pink to red colour denotes a positive reaction.

Reagent

The Microexpress® Rapid Test Kit for *E. coli* is a reagent set for laboratory use only.

The Microexpress® Rapid Test Kit for *E. coli* comprises of:

1. 10 vials containing 1 mL medium each for galactosidase activity and indole test.

Additional Material Required

0.9% Saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tube, incubator/water bath at 35°C-37°C.

Directions

Preparation of Inoculum

1. Isolate the organism to be identified on Brain Heart Infusion Agar (BHI).
2. Pick up a single well-isolated colony and streak on to BHI agar slant for enrichment and incubate at 37°C for 18-24 hours.
3. Observe for good growth.
4. Wash the growth with 2-3 mL sterile saline.
5. Match the turbidity of this suspension to McFarland Standard Number 0.5.

Inoculation of Vials

1. Bring the medium/vial to room temperature.
2. Inoculate the vial with 100 μ L of the above prepared inoculum.
3. Incubate at 37°C for 18-24 hours.
4. Observe for growth and colour development.
5. To perform Indole test, add 2-3 drops of Kovac's Reagent to the vial.

Quality Control

Appearance: Clear, colourless medium.

Cultural Response: Vials are inoculated with 100 μ L culture suspension of following organism, incubated at 30°C-35°C for 18-24 hours and results observed by adding 2-3 drops of Kovac's reagent at the end of incubation.

Organism (ATCC)	Results (Indole Test)	β -Galactosidase Activity
<i>Escherichia coli</i> (25922)	+	Yellow Colour
<i>Klebsiella aerogenes</i> (13048)	-	No colour change

Key: + = Red coloured ring in the alcohol layer - = No change in colour

Interpretation of Results

1. Development of yellow colour in the medium indicates positive test for galactosidase activity. A pink to red colour on addition of Kovac's Reagent is indicative of a positive test for *E. coli*.
2. No colour development denotes a negative test.

Remarks

1. The Micropress® Rapid Test Kit for *E. coli* is an *In vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Micropress® Rapid Test Kit for *E. coli* cannot be used directly on clinical specimens.
3. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes
4. Other coliforms may also be β -galactosidase positive. Pathogenic strain of *E. coli* O157:H7 is β -galactosidase negative and Indole positive.
5. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
6. Always use pure culture and a heavy inoculum for testing.
7. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of gram reaction, morphology, and other biochemical and serological tests.

Storage and Stability

1. Store the Micropress® Rapid Test Kit for *E. coli* in a cool, dry place at 2°C-8°C away from bright light.
2. Stability of the Micropress® Rapid Test Kit for *E. coli* is as per the expiry date mentioned on the label.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Godsey, J.H *et al.*, 1981. Rapid identification of Enterobacteriaceae with microbial enzyme activity profiles. J. Clin. Microbiol; 13: 483 -490.
2. Practical Medical Microbiology, Mackie & McCanney, 13th edition 1989, Edited by J. G. Coffee, J. P. Duguid. Standard Methods for The Examination Of Water And Wastewater, APHA, 20th Edition.
3. Spot Indole Test: Evaluation of Four Reagents, Journal of Clinical Microbiology, Apr. 1982, Vol. 15, No. 4, p: 589-592.
4. Data on file: Micropress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203180180001	Ready Prepared Kit	1 Kit (10Tests)

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.