Rapid Presumptive Identification Test Kit for E. coli

Intended Use
Rapid Presumptive Identification Test Kit for *E. coli* is used for fluorescence detection of *E. coli* on the basis of enzyme substrate reaction.

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, resulting in the release of the chromogen, and possessing the enzyme D-glucuronidase, which cleaves a fluorogenic substrate, resulting in the release of the fluorogen.

Principle
E. coli can be presumptively identified by the production of glucuronidase (GUD) which has been proposed as an alternative for IMViC tests (92-95% of *E. coli* possess β-glucuronidase). The media contains all nutrients required for the growth of coliforms and *E. coli* along with the substrate 4-methylumbelliferyl-D-glucuronide (MUG). The non-fluorescent substrate is cleaved by the enzyme GUD produced by *E. coli* to release the fluorogen (4-methylumbelliferone), which exhibits a bluish fluorescence when exposed to long wave (366 nm) ultraviolet light.

Reagent
The Microxpress® Rapid Presumptive Identification Kit for E. coli is a reagent set for laboratory use only.
The Microxpress® Rapid Presumptive Identification Kit for E. coli comprises of:
1. 10 vials containing mL medium each for glucuronidase activity.

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 µL of the above prepared inoculum.
3. Incubate at 37°C for 18-24 hours.
4. Observe for growth.
5. Observe for fluorescence under long wave UV light (340-380 nm).

Quality Control
Appearance: Clear, colourless medium.
Cultural Response: Vials are inoculated with 100 µL culture suspension of following organism, incubated for 18-24 hours at 30°C-35°C.

Organism (ATCC)	**Fluorescence**
Escherichia coli (25922)	+ (Blue green)
Klebsiella aerogenes (13048)	-

Note: Fluorescence observed under long wavelength (365 nm) UV light.

Interpretation of Results

1. Development of blue-green fluorescence indicates positive test for *E. coli*.
2. No fluorescence denotes a negative test.

Remarks

1. The Microxpress® Rapid Presumptive Identification Kit for E. coli is an In vitro diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Microxpress® Rapid Presumptive Identification 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. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly.
5. Good laboratory practices and hazard precautions must be observed at all times.
6. Always use pure culture and a heavy inoculum for testing.
7. Few strains of *Salmonella*, *Shigella*, *Pseudomonas*, Clostridia, Staphylococci produce this enzyme too.
8. Mostly β-glucuronidase activity occurs within 6 hours, but some weakly β-glucuronidase positive strains require overnight incubation.
9. 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 Microxpress® Rapid Presumptive Identification Kit for E. coli in a cool, dry place at 2°C-8°C away from bright light.
2. Stability of the Microxpress® Rapid Presumptive Identification 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. Berg J.D and L. Fiksdal (1988) Rapid detection of total and feacal coliforms in water by enzymatic hydrolysis of 4-methylumbelliferyl-D-glucuroside Appl. Environ. Microbiol: 54, 211 8-2122.
2. US-FDA Bacteriological analytical manual 8th ed. Revision A, AOAC International Gaithersburg.
3. Hama, AA, Perry. C.A Comparative study of presumptive and Confirmalive media for bacteria of the coliform group and for feacal streptococci- Am. J. Publ. Hl 33 550 556(1943).
4. Standard Methods for The Examination Of Water And Wastewater, APHA. 20th Edition.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203180170001	Ready Prepared Kit	1 Kit (10Tests)