



**Verotoxin Microwell ELISA**  
**For the rapid detection of Verotoxin producing *E. coli* in food samples**  
**Directions For Use**  
**For Laboratory Use Only**

**“When given the choice...  
take the SafePath”**

**Catalog Code: VTM-96 (96 Test)**

Supplying the World with Quality Diagnostics for  
Food Safety & Animal Health

## Intended Use

The SafePath™ *E. coli* Verotoxin assay is an enzyme-linked immunosorbent assay (ELISA) that may be used to screen food products for the presence of Verotoxin.

## Summary

The Verotoxin (VT), or shiga-like toxin family is a group of closely related toxins produced by certain strains of *Escherichia coli*.<sup>4</sup> These strains are a significant cause of human hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).<sup>1,3,7-8,12</sup> In addition, they are both water borne and food borne and may also be transmitted from person-to-person by the oral-fecal route.<sup>2</sup> In adults, illness caused by Verotoxin may last several days, while in children and the elderly, the illness can be fatal.<sup>2,11</sup>

Infections occur as sporadic illnesses or outbreaks, and are distributed throughout North and South America, Europe, and Asia.<sup>4,11</sup> The peak incidence of infection occurs during summer months.<sup>4</sup>

In the past, lengthy and cumbersome diagnostic procedures have been utilized for the detection of verotoxin.<sup>3-5,8,10</sup> Currently, simple to perform enzyme-linked immunosorbent assays (ELISA) are being used as an alternative to these longer tedious methods.<sup>5-6,9-10</sup> These antigen-detection assays provide a cost effective tool for the management of diarrheal disease with an accurate and rapid test.<sup>6,9-10</sup>

## Principle of Procedure

During the first incubation, Verotoxin antigen in the sample is captured by antibodies attached to the wells. The second incubation adds an additional anti-Verotoxin antibody cocktail that “sandwiches” the antigen. The next incubation attaches horseradish peroxidase to the sandwich. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

## Reagents

- Test strips: microwells containing rabbit anti-Verotoxin (VT1 &VT2) antibodies- 96 test wells.
- Test strip holder: One (1)
- Negative Control: One (1) vial containing 2 ml of a buffered base.
- Positive Control: One (1) vial containing 2 ml of inactivated Verotoxin antigen in a buffered base.

- Reagent 1: One (1) bottle containing 11 ml of monoclonal anti-Verotoxin antibodies in a buffer with preservative.
- Reagent 2: One (1) bottle containing 11 ml of an anti-mouse antibody conjugated to horseradish peroxidase in a buffer with preservative.
- Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
- Wash concentrate solution (20X): Two (2) bottles containing 25 ml of concentrated buffer and surfactant with preservative.
- Stop solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

Additional Materials Required:

- Stomacher (Tekmar stomacher lab-blender 400) or blender
- Shaking or similar incubator
- Microelisa plate reader capable of reading bichromatically at 450/650 nm (optional)
- Incubator, 37 °C
- Pipetter, 100 µl
- Disposable micropipette tips
- Microbiological media and antibiotics for preparation of necessary enrichment broths and plating media:
  - Novobiocin (Sigma N1628)
  - Modified EC broth (BBL #11187 or Difco #0314-01-0)
- Appropriate containers for storage and disposal of materials potentially contaminated with infectious agents
- Data record sheets
- Disinfecting Solution

### Precautions

Do not use solutions if they precipitate or become cloudy.

Exception: Wash concentrate may precipitate during refrigerated storage but will dissolve upon warming.

Do not add azides to the samples or any of the reagents.

Controls and some reagents contain a preservative.

Treat all reagents and samples as potentially infectious materials.

## Storage Conditions

Reagents, strips and bottled components:

Store between 2 – 7 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

## Reagent Preparation

### Wash Buffer

Remove cap and add contents of one bottle to 475 ml DI water. Transfer contents of diluted wash buffer into a squeeze bottle (small tip bottle).

## Medium Preparation

### Modified EC broth with Novobiocin (mEC+n)

1. Combine the following components with 1 liter of distilled water (if prepared media is not used):

Tryptone 20.0 g

Lactose 5.0 g

K<sub>2</sub>HPO<sub>4</sub> 4.0 g

KH<sub>2</sub>PO<sub>4</sub> 1.5 g

NaCl 5.0 g

Bile Salt #3 1.5 g                      pH adjusted to 6.9 ± 0.1

2. Autoclave at 121 °C for 15 minutes.

3. Allow to cool to room temperature and add 1 ml of a filter sterilized aqueous solution of 20 mg/ml Novobiocin. (for 225 ml add 0.225 ml of Novobiocin solution.) Final concentration should be 20 µg/ml.

## Sample Preparation

1. Add 225 ml of mEC+n to 25 g food product in a sterile stomacher bag or blender jar.
2. Stomach or blend sample and broth for 2 minutes.
3. Transfer stomacher bag to shaker at 37 °C or alternatively transfer contents of blender jar to a sterile flask and attach to shaker.
4. Incubate stomacher bags or flasks at 37 °C with shaking (120 rpm) for 18 hours.
5. Remove a 1 ml aliquot from each sample and place in a separate clean screw top test tube. This is the sample that will be used in the assay.

## Test Procedure

1. Break off number of wells needed (number of samples plus 2 for controls) and place in strip holder.
2. Add 100 µl of the negative control to well #1 and 100 µl of positive control to well #2 (use both as undiluted).
3. Add 100 µl of the test supernatant to the appropriate test well.
4. Incubate at room temperature for 30 minutes, then wash. \*
5. Add 2 drops of Reagent 1 (blue solution) to each well.
6. Incubate at room temperature for 30 minutes, then wash.
7. Add 2 drops of Reagent 2 (red solution) to each well.
8. Incubate at room temperature for 30 minutes, then wash. **Slap out excess fluid against an absorbent towel.**
9. Add 2 drops of Chromogen to each well.
10. Incubate at room temperature for 10 minutes.
11. Add 2 drops of Stop Solution to each well. Mix wells by tapping strip holder.
12. Read results visually or on a spectrophotometer using a bichromatic reading, with the filters set at 450nm and 620-650nm. Zero the reader on air.

\* Each washing consists of dumping the contents of the wells into an appropriate container with disinfecting solution (e.g. 3% bleach in water) and using the diluted wash buffer to fill to overflowing in each well, shaking out the contents and refilling the wells for a total of 3 times. Samples with sticky particulate matter may require more thorough washing than other samples. The potential exists for false positive results if the sample is not thoroughly washed from the well before addition of subsequent reagents.

Controls must be included each time the kit is run

### Interpretation of Results - Visual

**Reactive:** Any sample well that has distinct and substantial yellow color.

**Negative:** Any sample well that does not have distinct yellow color.

**NOTE:** The negative control, as well as some samples, may show some slight color.

## Interpretation of Results - ELISA Reader

Zero reader on air. Read all wells using a bichromatic reading with filters at 450nm and 620-650nm.

**Reactive:** Absorbance reading of 0.15 and above indicates the sample contains Verotoxin antigen.

**Negative:** Absorbance reading less than 0.15 indicates the sample does not contain detectable levels of Verotoxin antigen.

## Quality Control

The use of a positive and negative control allows easy validation of kit stability. For a valid test, the positive control must be over 0.5 OD units and the negative control must be under 0.15 OD units. Should the values fall outside these ranges, the kit should not be used.

## Troubleshooting

**Problem:** Negative control has substantial color development.

**Correction:** Washings were insufficient. Repeat test with more vigorous washings.

## References

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