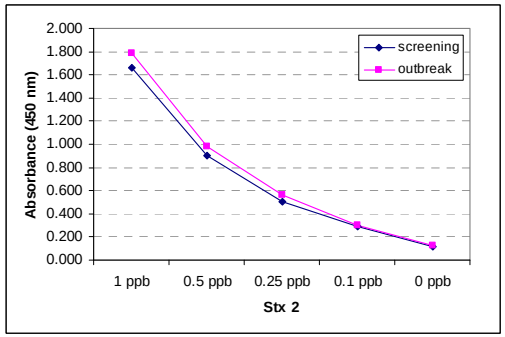
Importance of STEC Determination

Shiga toxin-producing *E. coli* (STEC) are food-borne pathogens that cause gastrointestinal illness, hemorrhagic colitis, and hemolytic uremic syndrome. As few as 100 cells can cause disease leading to serious illness or death. *Escherichia coli* O157:H7 is the STEC serotype most often implicated in outbreaks; however, there are numerous other STEC serotypes that have caused serious human illness and outbreaks. STEC that cause human illness were added as notifiable pathogens to the Nationally Notifiable Diseases Surveillance System in 2000. From 2000-2010, 7695 cases were reported; 5688 were associated with the serogroup O157, and the 83% of the other STEC were serogroups O26, O45, O103, O111, O121 and O145. However, other serogroups, including O91, O113, O104, have also caused serious human illness. *Escherichia coli* from each of the serogroups listed above have been found in ground beef, cattle hides, and in feces. Bovine feces can be a source of environmental contamination (soil, water) which can lead to secondary contamination of produce growing in fields.

It is difficult to distinguish pathogenic *E. coli* strains from non-pathogenic *E. coli* strains because the former rarely possess any distinguishing phenotypic or biochemical characteristics which differ from the latter. Methods such as the Shiga toxin ELISA described in this User's Guide have been developed to determine the presence of virulence markers, and therefore the presence of pathogenic *E. coli.* Raid, sensitive detection that can distinguish between Stx 2 and Stx 1 toxin types are vital for proper diagnosis.



Example of absorbances generated by different concentrations of Stx 2 in media incubated according to section D. Not intended for use in sample evaluation.

References

USDA-FSIS Microbiological Laboratory Guidebook Chapter 5.08 "Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges"

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India

Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444

Email: customerservice@lifetechindia.com, www.atzlabs.com ; www.lifetechindia.com

Life Technologies™
Your Molecular & Cell Technology Partner

ABRAXIS

Shiga toxin 2 ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Shiga toxin 2 (Stx 2) in Ground Beef Samples

Product No. 542010

1. General Description

The Abraxis Shiga toxin 2 ELISA is an immunoassay for the qualitative screening of Shiga toxin 2 (Stx 2). This test is suitable for the qualitative screening of Stx 2 in samples (please refer to the appropriate technical bulletins for additional matrices procedures). Shiga toxin is a virulence marker for O157 and non-O157 Shiga toxin producing *Escherichia coli* (STEC). This ELISA allows for the rapid and sensitive detection of Stx 2 and when used in combination with Stx 1 ELISA allows the differentiation of the two toxin types.

2. Safety Instructions

The positive control in the test kit contains small amounts of Shiga toxin 2. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with skin, wash with water. Excess beef and incubated media should be considered biological waste, and decontaminated, along with any contaminated equipment, by autoclaving or another effective method.

3. Storage and Stability

The Stx 2 ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is an indirect ELISA based on the recognition of Shiga toxin 2 by specific antibodies. Ground beef samples are incubated with a medium that encourages *E. coli* growth, if present, and shiga toxin production. If Stx 2 is present in a sample, is bound by an antibody immobilized on the wells of the microtiter plate. After a washing step, a second antibody is added which binds to the Stx 2 bound to the wells. A second washing step is followed by the addition of an HRP labeled antibody which binds to the existing antigen/antibody complex in the wells. After a final wash and addition of the substrate solution, a color signal is generated. The intensity of the blue color is proportional to the concentration of Stx 2 present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The result of the evaluation is compared to a known value to determine whether the sample is positive or below the limit of Stx 2 detection.

5. Limitations of the Stx 2 ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects cannot be completely excluded.

Samples must be diluted and incubated as instructed in the sample preparation section (Section D) or appropriate technical bulletin before testing in the ELISA.

Mistakes in handling the test also can cause errors. Possible sources for such errors include: improper storage of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reactions, extreme temperatures (lower than 10°C or higher than 30°C) during the test performance.

The Abraxis Stx 2 ELISA kit provides screening results only and does not differentiate between shiga toxin producing bacteria. Any positive results should be confirmed by PCR or another approved method.

A. Reagents and Materials Provided

1. Microtiter plate (8 wells X 12 strips) coated with a capture antibody, in a resealable aluminum pouch
2. Stx 2 positive control as Stx 2 (1): 0.5 ng/mL (ppb), X mL
3. Stx 2 negative control, X mL
4. Anti-Stx 2 Antibody Solution, X mL
5. Secondary antibody HRP Conjugate, X mL
6. Wash Solution (5X) Concentrate, 100 mL, must be diluted before use, see Test Preparation (Section C)
7. Substrate (Color) Solution (TMB), X mL
8. Stop Solution, X mL (handle with care)

B. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable sterile plastic tips (20-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with disposable sterile plastic tips (50-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Sterile clear polypropylene bags with mesh (ca. 24" x 30-36")
5. Stomacher (optional)
6. Modified tryptone soya broth with Novobiocin (mTSB+n) or other approved sample incubation medium
7. *E. coli* positive control (for inoculating media)
8. Incubator, static 42 ± 1°C
9. Automatic pipettor with 25 mL sterile disposable serological pipets
10. Paper towels or equivalent absorbent material
11. Timer
12. Autoclave
13. 50 mL sterile disposable conical vials

C. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. In order to equalize the incubation periods on the entire microtiter plate, a multi-channel pipette or a stepping pipette is recommended for adding the enzyme conjugate, antibody, substrate, and stop solutions. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, antibody, conjugate, substrate and stop solutions are ready to use and do not require any further dilutions.
4. Dilute the Wash Solution (5X) Concentrate at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water and mix thoroughly.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

D. Sample Preparation

Beef Samples

(see also USDA FSIS MLG Chapter 5.08: Detection, Isolation and Identification of *Escherichia coli* O157:H7 from Meat Products and Carcass and Environmental Sponges)

NOTE: the Abraxis Stx 2 ELISA may be used with either beef screening samples (sample diluted 1:4 in media) OR outbreak related samples (sample diluted 1:10 in media)

1. Disinfect surface of sample packaging prior to opening
2. i. Screening samples: Prepare in a sterile strainer bag a 1:4 dilution (one part beef in three parts media, e.g. 25 g beef with 75 mL enrichment medium). Increase or decrease total volume incubated according to amount needed to prepare representative samples and perform all analyses.
   ii. Outbreak related samples: Prepare in a sterile strainer bag a 1:10 dilution (one part beef in 9 parts media, e.g. 10 g beef with 90 mL enrichment medium). Increase or decrease total volume incubated according to amount needed to prepare representative samples and perform all analyses.
3. Pummel or hand massage until clumps are dispersed.
4. The USDA requires that both a known positive sample and an uninoculated medium control are incubated alongside each group of samples.
5. Incubate all bags for 15-22 hr at 42 ± 1°C.
6. After removing samples from incubator, mix gently by hand massaging to ensure uniformity and draw off 10-20 mL media from opposite side of mesh from beef sample.
7. Allow to settle 10-15 minutes in 50 mL conical vial. Gently decant top layer to a clean vial if desired, use as sample for ELISA. No further dilution is necessary.

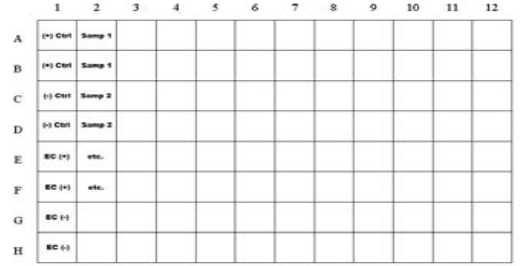
E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The FDA requires the incubation of O157 inoculated and uninoculated media alongside beef samples; positive and negative controls must be run with each test. Never use the values of controls that have been determined in a test performed previously.

(+) Ctrl, (-) Ctrl: kit controls

EC (+), EC (-): incubated positive and negative controls

Samp 1, Samp 2: incubated samples



F. Assay Procedure

1. Add 100 µL of the control solutions or samples (Section D) into the wells of the test strips according to the working scheme given. Analysis in duplicate or triplicate is recommended. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents.
2. Incubate for 60 minutes at room temperature.
3. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels
4. Add 100 µL of Anti-Stx 2 antibody solution to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents.
5. Incubate 30 minutes at room temperature.
6. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels
7. Add 100 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents.
8. Incubate 30 minutes at room temperature.
9. Remove the covering and decant the contents of the wells into a sink. Wash the strips three times using the diluted washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well in each washing step. Remaining buffer in the wells should be removed by patting the inverted plate dry on a stack of paper towels.
10. Add 100 µL of substrate (color) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 60 seconds. Be careful not to spill the contents. Incubate the strips for 20 minutes at room temperature. Protect the strips from direct sunlight.
11. Add 50 µL of stop solution to the wells in the same sequence as for the substrate solution.
12. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of stopping solution.

G. Evaluation

The evaluation of the ELISA can be performed by comparing the raw absorbance value for each control and sample, averaged between the two (or three) replicates, to the following known values. For both dilutions, raw absorbance (averaged between replicates) values of the kit positive control should range from 0.888 to 1.332; values for the kit negative control should range from 0.108 to 0.162. Absorbance values outside this range may indicate an invalid test. Uninoculated media, incubated without dilution alongside samples per USDA guidelines, should return raw absorbance values (averaged between replicates) ranging from 0.147 to 0.220. Values outside this range may indicate contamination.

Screening dilution: for samples incubated using the 1:4 dilution, absorbance values higher than 0.205 should be considered positive and submitted to further testing. Sensitivity using this method is as low as 30 pg/mL (approximately n cells per 25 g beef).

Outbreak Related dilution: for samples incubated using the 1:10 dilution, absorbance values higher than 0.215 should be considered positive and submitted to further testing. Sensitivity using this method is as low as 30 pg/mL (approximately n cells per 10 g beef).