

Shiga Toxin E. coli Microplate Assay

See package insert for more information

Preparations of Reagents:

Before use, bring all reagents to room temperature and mix gently. Return reagents to the refrigerator after use.

All reagents, except the Wash Buffer are supplied ready-to-use in dropper bottles. Dilute 10x Wash Buffer concentrate to 1x by adding 1 part concentrate to 9 parts distilled or deionized water. Diluted Wash Buffer is stable for 1 month when stored at 2-8°C.

Specimen Collection and Storage:

Fresh specimens (or specimens in Cary-Blair transport media) should be stored at 2-8°C or frozen at -20°C or lower immediately after collection. For direct testing of stool specimens, optimal results will be obtained if stools are tested immediately upon receipt in the laboratory. Specimens for culture should be put into broth within one to two hours after receipt in the laboratory.

Specimen Preparation For Assay:

Direct Stool Test:

1. Add 0.6mL Bacterial Specimen Diluent to a clean 12 x 75mm tube.
2. Mix stool as thoroughly as possible and dilute as follows:
 - Liquid or semi-solid stools: add 0.3mL (third mark from the tip of the pipette provided) using a transfer pipette. Thoroughly mix stool into the Bacterial Specimen Diluent and leave the transfer pipette in the tube.
 - Solid stools: add 0.3g (approximately 6mm diameter) using an applicator stick. Emulsify stool in the Bacterial Specimen Diluent and place a transfer pipette in the tube.
 - Stools in Cary-Blair transport media should be added directly into the microplate well without further dilution.
 - Vortex stool suspension.

Broth Method:

1. Inoculate 50µL or 50µg (small pea size) fresh stool or stool in Cray-Blair transport media into 5mL Tryptic Soy Broth (TSB), modified Tryptic Soy Broth (mTSB) or MacConkey Broth.
2. Incubate at 37°C for 18-24 hours.
3. Add 0.6mL Bacterial Specimen Diluent to a clean 12 x 75mm tube.
4. Transfer 0.3mL broth culture into a 0.6mL Bacterial Specimen Diluent using a transfer pipette. Leave the transfer pipette in the tube.

Test Procedure:

1. Open the foil pouch, remove the required number of microplate strips and place in strip holder. Use one well for the Negative Control and one well for the Positive Control. If using less than 8 wells, break off the required number of wells from

the strips. RETURN UNUSED MICROWELLS TO THE POUCH AND SEAL TIGHTLY TO EXCLUDE MOISTURE.

2. Add 4 drops of Negative Control to the first well. Add 4 drops of Positive Control to a second well.
3. Using a transfer pipette, add 4 drops of diluted specimen or undiluted stool in Cary-Blair transport media per well. **Note:** Place the opening of the transfer pipette just inside the wells to avoid splashing into adjacent wells.
4. Cover the microplate and incubate at room temperature for 60 minutes. Begin timing after the addition of the last specimen.
5. Shake out or aspirate the contents of the wells. Wash by completely filling each well with diluted Wash Buffer. Shake out or aspirate all fluid from the wells after each wash. Wash a total of 3 times. After the last wash, dump out contents and bang on clean paper towels or aspirate. Remove as much Wash Buffer as possible but do not allow the wells to dry at any time.
6. Add 4 drops of Enzyme Conjugate to each well.
7. Cover the microplate and incubate at room temperature for 30 minutes.
8. Decant or aspirate and wash each well 5 times as in step 5.
9. Add 4 drops Color Substrate to each well.
10. Cover the microplate and incubate at room temperature for 10 minutes.
11. Add 1 drop of Stop Solution to each well. Gently tap or vortex the wells until the yellow color is uniform. Read reactions within 10 minutes after adding Stop Solution. Read visually.

Quality Control:

Positive and Negative Controls must be included each time the test is performed. The Negative Control should be colorless when read visually. If yellow color equal to 1+ or greater on the Procedure Card is present in the Negative Control, the test should be repeated with careful attention to the wash procedure. Visually the color in the Positive Control should be equal to or greater than the 2+ reaction on the procedure card.

Visual Results:

1. Refer to the color card for the Microplate assay when interpreting results.
2. Read the Negative Control. The reaction should be colorless. If yellow color equal to 1+ or greater is present, the test should be repeated with careful attention to the wash procedure.
3. Read the Positive Control well. The intensity of color in the Positive Control should be equal to or greater than the 2+ reaction on the procedure card.
4. Read the test results by comparing with the reaction colors on the procedure card.
 - **Negative:** colorless
 - **Indeterminant:** faint yellow color, less than the 1+ reaction
 - **Positive:** yellow color of at least 1+ intensity
5. Interpretation of visual results:
 - **Negative:** A colorless reaction is a negative result and indicates that no Stx1 or Stx2 or an undetectable level of toxins is present in the sample tested.
 - **Indeterminant:** If faint yellow color that is less than the 1+ reaction develops, the test is indeterminant. Indeterminant results should be repeated.

If the repeat test results are positive, the specimen is positive. If the repeat test results are negative, the specimen is negative. If the repeat test results remain indeterminate, another specimen should be obtained and tested.

- **Positive:** If yellow color of at least 1+ intensity develops in the test well, the sample contains either Stx1 or Stx2 or both and the test is positive.