E. coli O157:H7 (E.coli) ELISA Kit

Catalog No.: abx050250

Size: 96T

Range: 6.25 × 10^3 cfu/ml - 4 ×10^5 cfu/ml

Sensitivity: < 1 ×10^3 cfu/ml

Storage: Store the 96-well plate, Standards at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of E.coli in food or water.

Introduction: Escherichia coli O157:H7 is an enterohemorrhagic serotype of the bacterium Escherichia coli and a cause of illness, typically through consumption of contaminated food. As an emerging pathogen, Escherichia coli O157:H7 causes severe enteritis and the extraintestinal complication of hemolytic-uremic syndrome. Rokhsartalab-Azar S et al evaluate the conjugate of E. coli O157: H7 lipopolysaccharide (LPS) with diphtheria toxoid (DT) as a candidate vaccine in mice model. Their results showed that the suggested vaccine composed of E. coli O157:H7 LPS and DT had a significant potential to protect against E. coli infections. Infection with Escherichia coli O157:H7 may develop into hemorrhagic colitis, or hemolytic uremic syndrome (HUS), which usually causes kidney failure or even death. The adhesion and toxins are the important virulent factors. Cai K et al constructed a novel vaccine candidate rSOBGs based on the bacterial ghost (BG). They found the novel vaccine candidate rSOBGs induced both anti-toxin and anti-adhesion immune protection, suggesting the possibility to prevent the infectious diseases caused by Escherichia coli O157:H7.

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-E.coli antibody is pre-coated onto a 96-well plate. HRP conjugated anti-E.coli antibody is used as a detection antibody. The standards, test samples and HRP conjugated antibody are added to the wells and washed with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the E.coli amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of E.coli can be calculated.

Kit components

1. One 96 well plate.
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 30 ml
4. Detection Reagent: (100X) 130 µl
5. Detection Reagent Buffer: 12 ml
6. TMB Substrate: 10 ml
7. Stop Solution: 10 ml
8. Wash Buffer (25X): 30 ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Food and water:** Incubate the sample for 12 hours at 37°C. Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Samples must be diluted so that the expected concentration falls within the kit's range.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Please bring sample slowly to room temperature.

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

General Sample guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration (4×10^6 × - 4×10^7 cfu/ml), dilute 1:100, for medium concentration (4×10^5 × - 4×10^6 cfu/ml), dilute 1:10 and for low concentration (6.25×10^3 - 4×10^5 cfu/ml), dilute 1:2. Very low concentrations (≤ 6.25 × 10^3 cfu/ml) do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

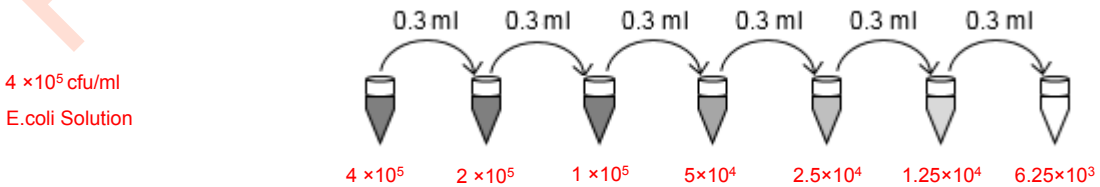
Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

3. Standard

The standard solution should be prepared no more than 2 hours prior to the experiment. Note: Do not dilute the standard directly in the plate. Two tubes of standard are included in each kit. Use one tube for each experiment.

a.) 4 ×10^5 cfu/ml of standard solution: Add 1 ml of Sample/Standard diluent buffer into one Standard tube to make the 4 ×10^5 cfu/ml solution. Keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles.

b.) 4 ×10^5 → 6.25×10^3 × standard solutions: Label 6 tubes with 2×10^5, 1×10^5, 5×10^4, 2.5×10^4, 1.25×10^4 and 6.25×10^3 cfu/ml. Aliquot 0.3 ml of the Sample /Standard diluent buffer into each tube. Add 0.3 ml of the above 4 ×10^5 × standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from the 1st tube to the 2nd tube and mix thoroughly. Transfer 0.3 ml from the 2nd tube to the 3rd tube and mix thoroughly, and so on.



Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.

4. Preparation of Detection Reagent working solution: prepare no more than 30 minutes before the experiment.
a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).
b.) Dilute the Detection Reagent with Detection Reagent Buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Detection Reagent into 99 µl of Detection Reagent Buffer.

B. Assay Procedure

The Detection Reagent working solution and TMB substrate must be kept warm at 37 °C for 30 min before use. It is recommend to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommend to measure each standard and sample in duplicate or triplicate.
2. Aliquot 0.1 ml of the diluted standards into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer into the control (zero) well.
4. Add 0.1 ml of appropriately diluted sample (food or water) into test sample wells.
5. Seal the plate with a cover and incubate at 37°C for 90 min.
6. Remove the cover and discard the plate content, tap the plate on absorbent filter papers. **Do not let the wells completely dry at any time.** Wash the plate 5 times with Wash buffer using one of the following methods:

Manual Washing: Discard the solution without touching the side walls. Fill each well completely (approximately 400 µl) with Wash buffer and incubate on an ELISA shaker for 2 min. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. Repeat this procedure for a total of five times.

Automated Washing: Discard the solution and wash the plate five times overfilling the wells with Wash buffer. After the final wash invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. It is recommended that the washer be set for a soaking time of 1-2 min.

7. Add 0.1 ml of Detection Reagent working solution into the wells (standard, test sample & zero wells). Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a cover and incubate at 37°C for 60 min.
9. Remove the cover and wash the plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.(See Step 6 for plate wash method).
10. Add 0.1 ml of TMB substrate into each well, cover the plate and incubate at 37°C in dark conditions for 15-30 min. (Note: This incubation time is for reference only, the optimal time should be determined by the end user.)
11. Add 0.1 ml of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation: (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The E. coli E.coli concentration of the samples can be interpolated from the standard curve.
Note: If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.Equilibrate the ABC working solution for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

C. Precautions

1. Before the experiment, centrifuge the tubes briefly to bring down any contents trapped in the lid.
2. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes. It is recommend to assay all standards, controls and samples in duplicate or triplicate.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use.
4. Do not let the plate dry out completely during the assay as this will inactivate the biological material on the plate. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. Do not use expired components or components from different batches.
9. The TMB Substrate solution is easily contaminated; work under sterile conditions when hanling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Typical Data & Standard Curve

Results of a typical standard run of a E. coli E.coli ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

Concentration (cfu/ml)	0	6.25×10^3	1.25×10^4	2.5×10^4	5×10^4	1×10^5	2×10^5	4×10^5
OD450	0.072	0.147	0.207	0.309	0.521	0.813	1.516	2.824