Escherichia coli O157:H7, E.coli O157:H7 ELISA Kit

Catalog No.: BEK1249

Size: 96T

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

Sensitivity < 1 ×10^3 cfu/ml

Specificity: No cross react with other cytokines

Storage and Expiration: Store at 2-8℃ for 6 months, or at -20℃ for 12 months. **Store HRP conjugated antibody at 2-8℃.**

Application: For quantitative detection of E.coli O157:H7 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 was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-E.coli O157:H7 monoclonal antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-E.coli O157:H7 polyclonal antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the E.coli O157:H7 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of E.coli O157:H7 can be calculated.

Kit components

1. One 96-well plate pre-coated with anti-E.coli O157:H7 antibody
2. Lyophilized E.coli O157:H7 standards: 2 tubes (4 × 10^5 cfu / tube)
3. Sample diluent buffer: 30 ml
4. HRP conjugated anti-E.coli O157:H7 antibody (Concentrated): 130 µl. Dilution: 1:100
5. Antibody diluent buffer: 12 ml
6. TMB substrate: 10 ml
7. Stop solution: 10 ml
8. Wash buffer: 30 ml (25x). Dilution: 1:25

Note: Reconstitute standards and test samples with Kit Component 3.

Material Required But Not Provided

1. Microplate reader (wavelength: 450nm)
2. Precise pipette and disposable pipette tips
3. Automated plate washer
4. ELISA shaker
5. 1.5ml of Eppendorf tubes
6. Plate cover
7. Absorbent filter papers
8. Plastic or glass container with volume of above 1L

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Protocol

- **Preparation of sample and reagents**

1. Wash buffer

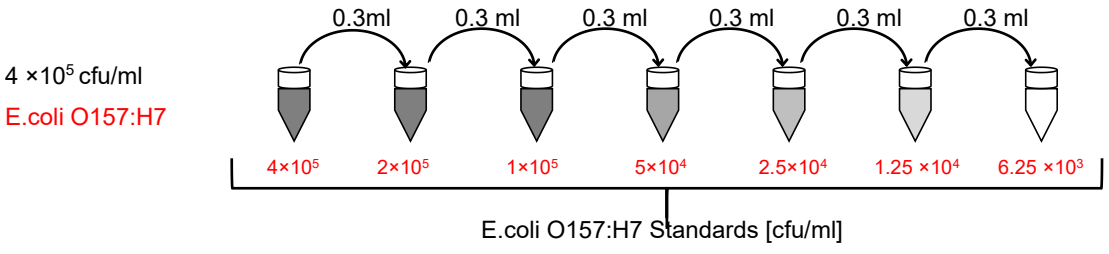
Dilute the concentrated Wash buffer 25-fold (1:25) with distilled water (i.e. Add 30 ml of concentrated wash buffer into 720 ml of distilled water).

2. Standard

Reconstitution of the lyophilized E.coli O157:H7 standard (Kit Component 2): standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. **(Note: Do not dilute the standard directly in the plate)**

a. 4 ×10^5 cfu/ml of standard solution: Add **1 ml** of Sample / Standard diluent buffer (Kit Component 3) into one Standard (Kit Component 2) tube, keep the tube at room temperature for 10 min and mix thoroughly.

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



Note: The standard solutions are best used within 2 hours. The 4 ×10^5 cfu/ml standard solution should be used within 12 hours. Or store at -20℃ for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of HRP conjugated anti-E.coli O157:H7 antibody (Kit Component 4) working solution: the HRP conjugated antibody should be used within 30 min after diluting.

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 HRP conjugated anti-E.coli O157:H7 antibody (Kit Component 4) with Antibody diluent buffer (Kit Component 5) at 1:100 and mix thoroughly. i.e. Add 1 µl of HRP conjugated anti-E.coli O157:H7 antibody into 99 µl of Antibody diluent buffer.

- **Assay procedure**

Equilibrate all kit components to room temperature before use.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommend to measure each sample, standard, zero and optional

control sample in duplicate. Remove extra microwell strips from holder and store them in foil bag at 2-8℃ or at -20℃ for long term.

2. Aliquot 0.1 ml of 4 $\times 10^5$ cfu/ml, 2 $\times 10^5$ cfu/ml, 1 $\times 10^5$ cfu/ml, 5 $\times 10^4$ cfu/ml, 2.5$\times 10^4$ cfu/ml, 1.25 $\times 10^4$ cfu/ml, 6.25 $\times 10^3$ cfu/ml standard solutions into the standard wells.
3. Add 0.1 ml of Sample / Standard diluent buffer (Kit Component 3) into the control (zero) well.
4. Add 0.1 ml of properly diluted sample (food or water) into test sample wells.

Here is an example of the arrangement of sample, standard and zero in the plate wells:

	1	2	3	4
A	Standard 1 (4 $\times 10^5$ cfu/ml)	Standard 1 (4 $\times 10^5$ cfu/ml)	Sample 1	Sample 1
B	Standard 2 (2 $\times 10^5$ cfu/ml)	Standard 2 (2 $\times 10^5$ cfu/ml)	Sample 2	Sample 2
C	Standard 3 (1 $\times 10^5$ cfu/ml)	Standard 3 (1 $\times 10^5$ cfu/ml)	Sample 3	Sample 3
D	Standard 4 (5 $\times 10^4$ cfu/ml)	Standard 4 (5 $\times 10^4$ cfu/ml)	Sample 4	Sample 4
E	Standard 5 (2.5 $\times 10^4$ cfu/ml)	Standard 5 (2.5 $\times 10^4$ cfu/ml)	Sample 5	Sample 5
F	Standard 6 (1.25 $\times 10^4$ cfu/ml)	Standard 6 (1.25 $\times 10^4$ cfu/ml)	Sample 6	Sample 6
G	Standard 7 (6.25 $\times 10^3$ cfu/ml)	Standard 7 (6.25 $\times 10^3$ cfu/ml)	Sample 7	Sample 7
H	Zero / Blank	Zero / Blank	Sample 8	Sample 8

5. Seal the plate with a cover and incubate at 37℃ for 90 min.
6. Remove the cover, aspirate the plate content and wash plate 5 times with Wash buffer (Kit Component 8) using one of the following methods:

   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 8) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure four more times for a **total of FIVE washes**.

   Automated Washing: Aspirate all wells, then wash plate **FIVE times** with Wash buffer (Kit Component 8) (overfilling wells with the buffer, about 400μl). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.
7. Add 0.1 ml of HRP conjugated anti-E.coli O157:H7 antibody work solution into each well. Add the solution at the bottom of each well without touching the side wall.
8. Seal the plate with a new cover and incubate at 37℃ for 60 min.
9. Remove the cover, aspirate the plate content and wash plate 5 times with Wash buffer (Kit Component 8), and each time let the wash buffer stay in the wells for 1-2 min. (Repeat Step 6).
10. Add 0.1 ml of TMB substrate (Kit Component 6) into each well, cover the plate and incubate at 37℃ in dark within 30 min. (**Note:** The color development on the plate should be monitored and the substrate reaction stopped before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay. It is recommended to add the stop solution when the highest standard has developed a dark blue color.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated E.coli O157:H7 standard solutions), the other wells show no obvious color.

11. Add 0.1 ml of Stop solution (Kit Component 7) into each well and mix thoroughly. The color changes into yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

Note: If the incubation without shaking, the obtained O.D. Values may be lower than the typical data, but the results are still valid.

For calculation, average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. (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). The E.coli O157:H7 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Precautions

1. All reagents should be considered as potentially hazardous. It is recommend that this kit is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
2. **Store HRP conjugated anti-E.coli O157:H7 antibody (concentrated) (Kit Component 4) at 2-8℃, do NOT store it at -20℃.**
3. Do not use expired components or mix components from different lots or suppliers.
4. Avoid contact of skin or mucous membranes with kit reagents or specimens.
5. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
6. Avoid contact of substrate solution with oxidizing agents and metal.
7. Avoid splashing or generation of aerosols.
8. To avoid microbial contamination or cross-contamination of reagents or specimens, it is recommended to use the clean and separate pipette tip for each.
9. Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent. Exposure to acid inactivates the conjugate.
10. Distilled or deionized water must be used for reagent preparation.
11. Substrate solution must be equilibrated at room temperature prior to use.

Typical Data & Standard Curve

Results of a typical standard run of a E.coli O157:H7 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. (N/A=not applicable)

X	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
Y	OD450	0.072	0.147	0.207	0.309	0.521	0.813	1.516	2.824

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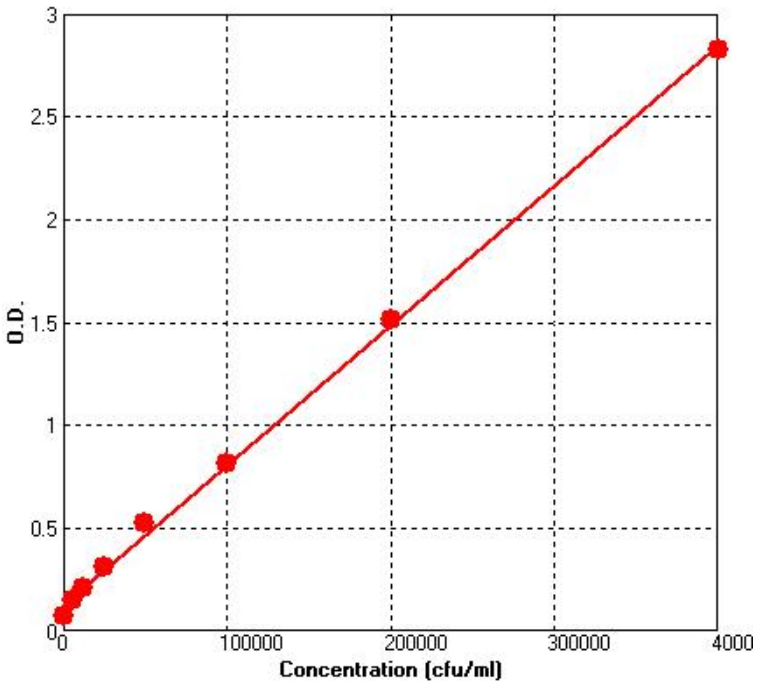

Reference

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3. Cai K, Tu W, Liu Y, Li T, Wang H. Novel fusion antigen displayed-bacterial ghosts vaccine candidate against infection of Escherichia coli O157:H7. Sci Rep. 2015 Dec 2;5:17479.

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