E. coli O157 ELISA Kit

ARG83598 E. coli O157 ELISA Kit is an Enzyme Immunoassay kit for the quantification of E. coli O157.

Catalog number: ARG53598

Package: 96 wells

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique for detection and quantitation of E. coli O157 in Food, Fecal or Environmental Samples. An antibody specific for E. coli O157 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any E. coli O157 present is bound on the plate. After washing away any unbound substances, a Horseradish Peroxidase (HRP) conjugated primary antibody binds to E. coli O157 is added to each well and incubates. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of total E. coli O157 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of total E. coli O157 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, the Standard should be aliquoted and stored at-20°C to avoid repeated freeze-thaw cycles. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard (5 x 10^8 CFU equivalents/mL, Heat Killed E. coli)	200 µl	-20°C
10X Wash Buffer	100 ml	4°C
1000X conjugated-E. coli O157 Antibody	20 µl	4°C
1000X HRP-Streptavidin	20 µl	4°C
Assay Diluent	50 ml (Ready-to-use)	4°C
TMB substrate	12ml (Ready-to-use)	4°C (Protect from light)
STOP solution	12ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620 nm as reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- Microplate shaker.
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, the Standard should be aliquoted and stored at -20°C to avoid repeated freeze-thaw cycles. Store all other components at 4°C.
- If crystals are observed in the 10X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

All Sample- Sonicate or homogenize Sample in E. coli O157 culture medium at 1:9 dilution factor. Incubate 18-24 hours at 37°C.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer, mix well. Storage at 2-8°C.
- **1X conjugated-E. coli O157 Antibody:** Dilute the antibody immediately before use; dilute the **1000X conjugated-E. coli O157 Antibody** concentrate into **Assay Diluent** to yield 1X conjugated-E. coli O157 Antibody. Do not store diluted solutions.
- **1X HRP-Streptavidin:** Dilute the reagent immediately before use; dilute the **1000X HRP-Streptavidin** into **Assay Diluent** to yield 1X HRP-Streptavidin. Do not store diluted solutions.
- **Sample**: If the assay found samples contain E. coli O157 higher than the highest standard (5 x 10^7 equivalent CFUs/mL), the samples can be diluted with Assay Diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended making series dilutions with Assay Diluent for each unknown sample to do pre-test to determine the suitable dilution factor).

- **E. coli O157 standard:** Prepare a series dilution of E. coli O157 standards with Assay Diluent. The Assay Diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Assay Diluent as according to the suggested concentration table below:

Standard No	E. coli O157 (10^4 equivalent CFUs/mL)	Assay Diluent (µl)	Standards (µl)
S1	5000	360	40 (5x 10^8 equivalent CFUs/mL stock)
S2	1250	300	100 µl (S1)
S3	313	300	100 µl (S2)
S4	78	300	100 µl (S3)
S5	20	300	100 µl (S4)
S6	4.8	300	100 µl (S5)
S7	1.2	300	100 µl (S6)
S0	0	300	0

Note: Dilutions for the standard must be made and applied to the plate immediately. S0 serves as background.

ASSAY PROCEDURE

Warm Substrate Solution to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it. Standards and samples should be assayed in duplicates.
2. Add **100 µl of the Standards** and **samples** into the appropriate wells. Incubate for **2 hours at room temperature** on a microplate shaker.
3. Aspirate each well and wash, repeating the process 4 times for a total **5 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting
4. Add **100 µl** of the **1X conjugated-E. coli O157 antibody** to each well, incubate for **1 hour at RT** on a microplate shaker.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of the **1X HRP-Streptavidin working solution** to all wells and incubate for **1 hour at RT** on a microplate shaker.
7. Aspirate each well and **wash as step 3**. Proceed immediately to the next step.
8. Add **100 µl** of **TMB substrate solution** into each well. Incubate for **2-30 mins at RT** on microplate shaker. Avoid exposure to light.
9. Add **100 µl** of **Stop Solution** to each well.
10. Read the OD with a microplate reader at **450 nm** immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and samples.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Assay Range

10^4 CFU equivalents/mL- 5 x 10^7 CFU equivalents /mL