Operation Manual

Product Name: E.coli Host Cell Protein ELISA Kit, G3 **Cat NO.:** EH-E0022-3-48T

*For the quantitative Measurement of E. coli HCP Residues **(6-Strain Combo)** in Cell Culture Supernatants, Protein Purification Process, and End-Product.*

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1. Materials (Note: Storage at 2-8℃)

	Reagents	Specification	Quantity
1	Pre-Coated Microplate (Detachable)	48 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500μg/mL	20μL	1 tube
3	Detection antibody (100×)	100μL	1 tube
4	TMB Substrates	10mL	1 vial (Avoid Light)
5	Stop Solution	10mL	1 vial
6	Wash Solution (100×)	10mL	1 vial
7	Diluent Buffer (10×)	10mL	1 vial
8	Plate Sealer		4 pieces
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2. Equipment Required But Not Provided

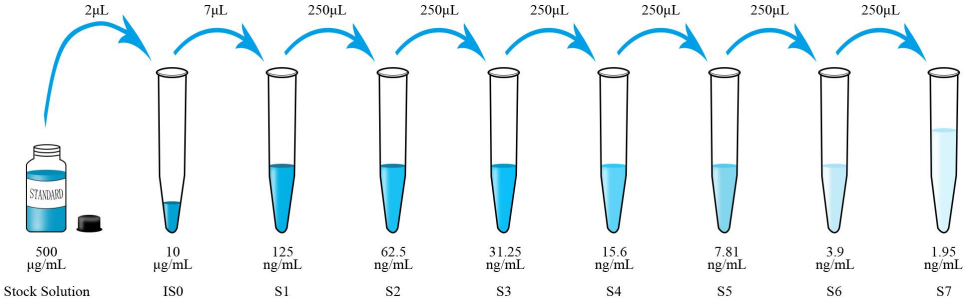
1	10-1000μL pipettor	**6**	Microplate reader
2	Multichannel pipettor	**7**	High-speed centrifuge
3	1L sterilized deionized water or ultrapure water	**8**	Mini Centrifuge
4	Sterilized EP tubes	**9**	Microplate washer or washing bottles
5	Absorbent Paper	**10**	Data analysis and graphing software

3. Kit's Preparation

1) Place all kits' components at RT for 30mins before using.
2) The preparation of Wash Solution (1×): Dilute 10 mL of Wash Solution (100×) with 990 mL of deionized water or ultrapure water to prepare 1000 mL of Wash Solution (1×).
3) The preparation of Diluent Buffer (1×): Dilute 10 mL of Diluent Buffer (10×) with 90 mL of deionized water or ultrapure water to prepare 100 mL of Diluent Buffer (1×).

4. Reagent Preparation

1) Remove the microplate, use the needed strips, reseal and store the remainder at 4°C.
2) Put the strips on a clean microplate frame (reusable) and allow them to return to RT before starting the experiment.
3) Prepare Standards: Label tubes IS0, S1–S7 and add Diluent Buffer (1×): IS0 (98μL), S1 (553μL), S2–S7 (250μL each). Add 2μL Standard (Stock Solution-500μg/mL) to IS0, mix well. Transfer 7μL IS0 to S1, mix. Perform a 2-fold serial dilution from S1 to S7 by transferring 250μL each time.

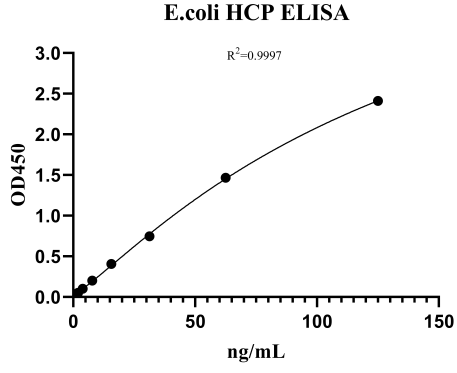


5. Assay Procedure

1) Add 100ul of diluted standards (S1-S7) or 100ul samples to appropriate wells. For the Blank Control test, add 100ul Diluent Buffer (1×) to the well.
2) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at RT.
3) Washing Step: (Automated Washing) Fill the automated microplate washer with 1000 mL Wash Solution (1×) and wash the microplate for 5 cycles, then invert and tap the plate on absorbent paper until dry. Or (Manual Washing) Fill each well with 300μL Wash Solution (1×), let stand for 20 seconds, then discard the contents and dry by tapping the plate onto absorbent paper. Repeat this step 5 times.
4) Prepare Detection Antibody: Extract 60ul of Detection antibody (100×), and add it into 6ml Diluent Buffer (1×) and mix gently. Add 100ul diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 90 mins at RT.
5) Washing Step: Repeat the same procedure as step 3.
6) Add 100ul TMB substrate to each well. Cover the plate with a sealer, incubate at RT for about 15 mins. If the color is light, the reaction time can be extended appropriately, but not more than 30min.
7) Add 50ul of Stop Solution to each well to stop the reaction.
8) Run the microplate reader and conduct measurement at 450nm.

6. Data Analysis

1) Calculate the average absorbance.(Standards/samples are recommended be run in duplicates).
2) Plot the average O.D. vs. Concentration and fit a **4-parameter logistic (4-PL)** curve.
3) Calculate the concentration of samples from the standard curve.
4) Standard curve is for demonstration only:



ng/mL	OD450
125	2.410
62.5	1.466
31.25	0.745
15.625	0.405
7.8125	0.201
3.90625	0.100
1.95	0.05