



Operation Manual

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

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°C)

	Reagents	Specification	Quantity
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500µg/mL	20µL	1 tube
3	Detection antibody (100×)	150µL	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
9	Instruction Manual		1

2. Equipment Required But Not Provided

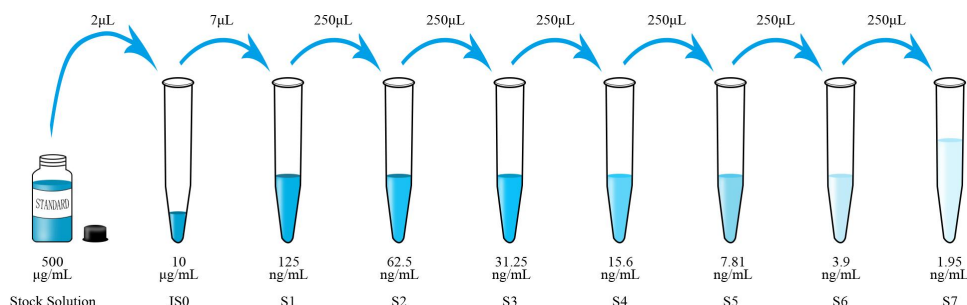
1	10-1000µL pipettor	6	Microplate reader
2	Multichannel pipettor	7	High-speed centrifuge
3	1L sterilized deionized water or ultrapure water	8	Mini Centrifuge
4	Sterilized EP tubes	9	Microplate washer or washing bottles
5	Absorbent Paper	10	Data analysis and graphing software

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.

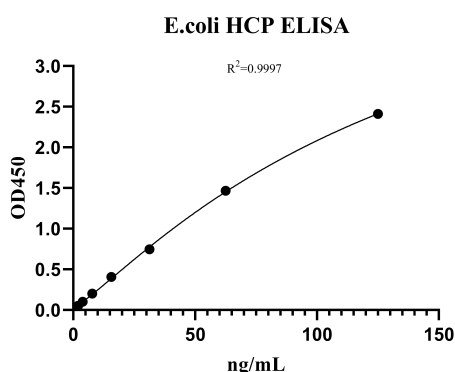


5. Assay Procedure

- 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 100ul of Detection antibody (100×), and add it into 10ml 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