

**Operation Manual****Product Name:** E. coli Host Cell Protein ELISA Kit, G3 **Cat NO.:** EH-E0020-3*For the quantitative Measurement of E. coli HCP Residues in Cell Culture Supernatants, Protein Purification Process, and End-Product**Scan to see the full manual***1. Materials** (Note: Storage at 2-8°C)

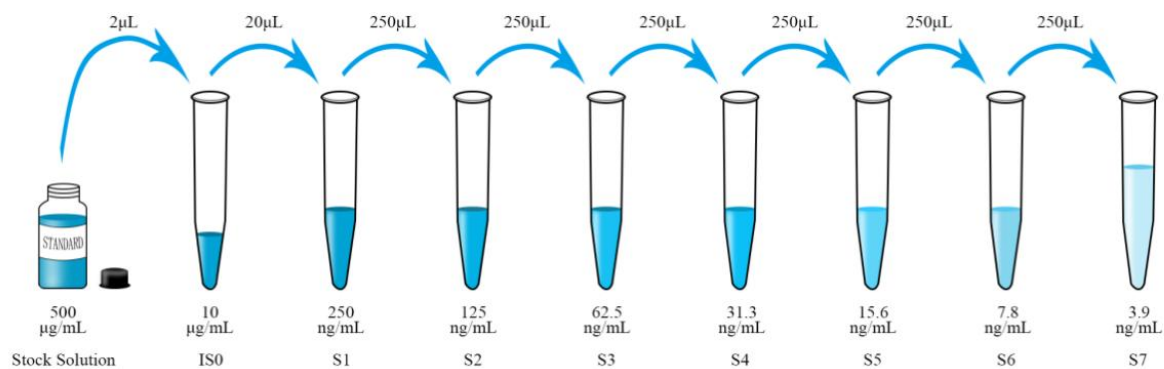
	Reagents	Specification	Quantity
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500ug/ml	50ul	1 tube
3	Detection antibody (100×)	150ul	1 tube
4	TMB Substrates	10ml	1 vial (Avoid Light)
5	Stop Solution	10ml	1 vial
6	Wash Solution (100×)	10ml	1 vial
7	Diluent Buffer (10×)	10ml	1 vial
8	Plate Sealer		4 pieces
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**2. Kit's Preparation**

- Place all kits' components at room temperature for 30mins before using.
- 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×).
- The preparation of Diluent Buffer: Dilute 10 mL of Diluent Buffer (10×) with 90 mL of deionized water or ultrapure water to prepare 100 mL of Diluent Buffer (1×).

**3. Reagent Preparation**

- Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C.
- Prepare Standards: When preparing the standards, label 8 tubes IS0, S1-S7 and add a certain volume of diluent: IS0 (98ul), S1 (780ul), S2 to S7 (each 250ul). Produce a 2-fold dilution series until S7 (see below).



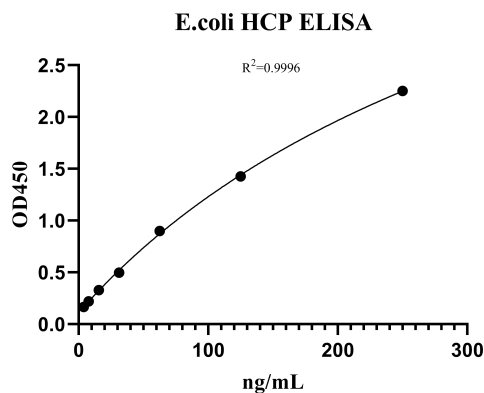


#### 4. Assay Procedure

- 1) Place the microplate strips on a clean microplate frame and allow them to return to room temperature before starting the experiment.
- 2) Add 100ul of diluted standards (S1-S7) or 100ul (diluted or undiluted) samples to appropriate wells. For the Blank Control test, we recommend add 100ul Diluent Buffer to the well.
- 3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at room temperature.
- 4) Washing Step: (Automated Washing) Fill the automated microplate washer with 1000 mL Wash Solution (1×) and wash the microplate for 5 cycles.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.
- 5) Prepare Detection Antibody: Extract 100ul of Detection antibody (100×), and add it into 9.9 ml Diluent Buffer to reach its working concentration (1×) and mix gently. Add 100ul of above 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 room temperature.
- 6) Washing Step: Repeat the same procedure as step 4.
- 7) Add 100ul TMB substrate to each well. Cover the plate with a sealer, incubate at room temperature for about 15 mins. If the color is light, the reaction time can be extended appropriately, but not more than 30min.
- 8) Add 50ul of Stop Solution to each well to stop the reaction.
- 9) Run the microplate reader and conduct measurement at 450nm.

#### 5. Data Analysis

- 1) It is recommended to use four-parameter curve fitting.
- 2) Calculate the average absorbance for each standard and sample. Standards and samples are recommended be run in duplicates.
- 3) Construct a standard curve by plotting the average O.D. against the concentration and generate a four-parameter logistic (4-PL) curve-fit.
- 4) Calculate the concentration of samples from the standard curve.
- 5) Standard curve is for demonstration only



ng/ml	OD450
250	2.251
125	1.427
62.5	0.898
31.25	0.496
15.625	0.329
7.8125	0.219
3.90625	0.166