Operation Manual

Product Name: Pichia Yeast Host Cell Protein ELISA Kit, G2 **Cat NO.:** PH-E0021-1-48T

For the quantitative Measurement of Pichia Yeast HCP Residues 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)	48 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500ug/ml	30ul	1 tube
3	Detection antibody (100×)	100ul	1 tube
4	TMB Substrates	6ml	1 vial (Avoid Light)
5	Stop Solution	6ml	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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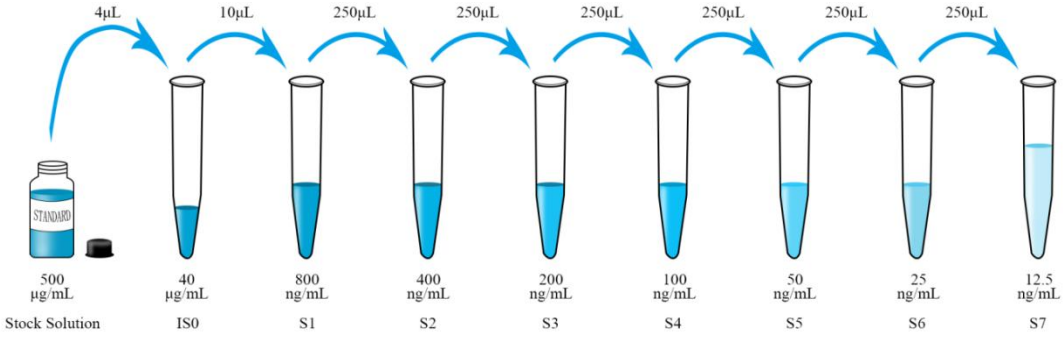
2. Kit's Preparation

1) Place all kits' components at room temperature 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: 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

1) 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.
2) Prepare Standards: When preparing the standards, label 8 tubes IS0, S1-S7 and add a certain volume of diluent: IS0 (46ul), S1 (490ul), S2 to S7 (each 250ul). Produce a 2-fold dilution series until S7 (see below).

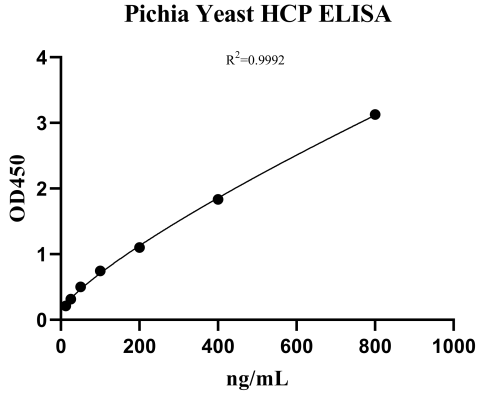


4. Assay Procedure

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 60ul of Detection antibody (100×), and add it into 6 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
800	3.127
400	1.836
200	1.102
100	0.745
50	0.501
25	0.315
12.5	0.212