# ab285247 – CHO Host Cell Proteins Elisa Kit

For the quantitative measurement of CHO HCP in Human serum, plasma and cell culture supernatants.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
http://www.abcam.com/ab285247

## Storage and Stability

The entire kit may be stored at 4°C protected from light for up to 12 months from the date of shipment. Avoid freeze-thaw cycles.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Micro ELISA Plate | 8 wells ×12 strips | 4°C |
| Detection Antibody | 11 mL | 4°C |
| Enzyme Conjugate | 11 mL | 4°C |
| Standard (S1) (100 ng/mL) | 1 mL | 4°C |
| Standard (S2) (33 ng/mL) | 1 mL | 4°C |
| Standard (S3) (11 ng/mL) | 1 mL | 4°C |
| Standard (S4) (3.6 ng/mL) | 1 mL | 4°C |
| Standard (S5) (1.2 ng/mL) | 1 mL | 4°C |
| Standard (S6) (0 ng/mL) | 1 mL | 4°C |
| Diluent | 100 mL | 4°C |
| TMB Substrate | 11 mL | 4°C |
| 20X Wash Solution | 50 mL | 4°C |
| Stop Solution | 6 mL | 4°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Incubator (37°C)
- Microplate reader capable of measuring absorbance at 450 nm

## Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Wash Solution: Dilute the wash solution concentrate (20X, 25 mL) to 1X in water.

Sample Dilution: Use Diluent (1x) provided in this kit to dilute samples if the CHO HCP concentration in the sample is beyond the detection range.

## Assay Protocol

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
- High or low pH, detergents, urea, high salt concentrations, and organic solvents are some of the known interference factors. pH can affect the assay.

1. Secure the desired numbers of coated wells in the holder then add 100 ul of Standards or Samples to the appropriate well in the antibody pre-coated Microtiter Plate. Cover and incubate the plate for 2 hours at 37°C.
2. Wash the microtiter plate using one of the specified methods indicated below:
   a) **Manual Washing:** Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with 1× wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears.
   **Δ Note:** *Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.*
   b) Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
3. Add 100 µL of Detection Antibody to each well. Mix well. Cover and incubate the plate for 1.5 hour at 37°C.
4. Wash the microtiter plate five times as stated in section 2a.
5. Add 100 µL of Enzyme conjugate to each well. Mix well. Cover and incubate the plate for 45 min at 37°C.
6. Wash the microtiter plate five times as stated in section 2a.
7. Add 100 µL Substrate Solution to each well including control wells, subsequently. Cover and incubate for 10-15 minutes at 37°C. (Avoid light).
8. Add 50 µL of Stop Solution to each well including control wells. Mix well.
9. Determine the Optical Density (O.D.) at 450 nm using a microplate reader immediately.

## Calculation

1. Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
2. Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve e using graph paper or statistical software to generate a linear regression, four parameters logistic (4-PL) curve-fit, or curvilinear regression of second degree. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
3. Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.
   **Δ Note:** The coefficient of determination of the standard curve should be ≥ 0.95 and the highest O.D. should be more than 1.0.

| Standards (ng/mL) | OD of duplicates | |
|---|---|---|
| 100 | 3.3324 | 3.4102 |
| 33 | 2.1084 | 2.078 |
| 11 | 1.4345 | 1.472 |
| 3.6 | 0.9979 | 0.8785 |
| 1.2 | 0.4633 | 0.4336 |
| 0 | 0.087 | 0.0795 |

## Assay Precision

| | Intra-Assay Precision | Inter-Assay Precision |
|---|---|---|
| CV (%) | 10.8 | 5.3 |

**Linearity:**

| Sample Dilution | Range (%) |
|---|---|
| 1:500 | 96 – 101 |
| 1:1000 | 93 – 107 |
| 1:2000 | 92 – 100 |
| 1:4000 | 96 – 108 |

**Cross-Reactivity:**

| Sample | Cross-Reactivity (%) |
|---|---|
| Vero HCP | 0.08 |
| Yeast HCP | 0.10 |
| E.coli HCP | 0.07 |

**Technical Support**



For all technical or commercial enquiries please go to:

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