Chinese Hamster Ovary (CHO) Host Cell Protein (HCP) Residue ELISA Kit

Catalog Number. CSB-EQ33262CHO

For the quantitative determination of Chinese Hamster Ovary (CHO) Host Cell Protein (HCP) concentrations in cell lysates.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: https://www.cusabio.com/

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for CHO HCP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for CHO HCP. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CHO HCP bound in the initial step. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

6.25 ng/ml-400 ng/ml.

SENSITIVITY

The minimum detectable dose of CHO HCP is typically less than 3.1 ng/ml.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of CHO HCP. No significant cross-reactivity or interference between CHO HCP and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between CHO HCP and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96 wells)
High Concentration Standard (500 µg/ml)	1 x 50 µl
HRP-conjugate (100 x)	1 x 150 µl
Wash Buffer (100 x)	1 x 10 ml
Sample Diluent (10 x)	1 x 10 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to one week.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- 10 µL-1000 µL multichannel micropipette.
- Sterilization deionized water or ultrapure water.
- Pipettes and pipette tips.
- Sterilization EP tubes.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 1 day may be stored at 2-8°C, otherwise samples must be aliquoted and stored at -20°C (<3 months) or -80°C (<6 months) to avoid loss of bioactivity and contamination.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
5. Samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
6. Owing to the possible interference (e.g.,complex buffer solutions produced during the purification of target), the result may not be good.
7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent.**
- Bring all reagents to room temperature before use for 30 minutes.
- Deionized water or ultrapure water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

1. Sample Diluent

Centrifuge the vial before opening. Sample Diluent requires a 10-fold dilution. A suggested 10-fold dilution is 10 ml of Sample Diluent (10 x) + 90 ml of deionized water or ultrapure water, mix well.

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved.

2. HRP-conjugate

Centrifuge the vial before opening. HRP-conjugate (100 x) requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate (100 x) + 990 µl of Sample Diluent, mix well.

3. Wash Buffer

If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 ml of Wash Buffer (100 x) into deionized water or ultrapure water to prepare 1000 ml of Wash Buffer, mix well.

4. Standard

Take 8 bottles for standard preparation, number: S0-S7, add 48 μl、490 μl、250 μl、250 μl、250 μl、250 μl、250 μl of **Sample Diluent** into corresponding tube.

Take 2 μl of **High Concentration Standard** (500 μg/ml) into first tube, shake well, the concentration of S0 is 20 μg/ml; then take 10 μl of S0 into second tube, shake well, the concentration of S1 is 400 ng/ml; then take 250 μl of S1 into third tube, shake well, the concentration of S2 is 200 ng/ml; then produced 2-fold dilution series until S7. Mix each tube thoroughly before the next transfer. (All the process as below)

Number	Sample Diluent	Add the solution into corresponding tube	Concentration
S0	48 μl	2 μl of High Concentration Standard	20 μg/ml
S1	490 μl	10 μl of S0	400 ng/ml
S2	250 μl	250 μl of S1	200 ng/ml
S3	250 μl	250 μl of S2	100 ng/ml
S4	250 μl	250 μl of S3	50 ng/ml
S5	250 μl	250 μl of S4	25 ng/ml
S6	250 μl	250 μl of S5	12.5 ng/ml
S7	250 μl	250 μl of S6	6.25 ng/ml

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a blank well with 100 µl of **Sample Diluent**. Add 100 µl of **Standard** or **Sample** per well. Standard need test in duplicate.
4. Mix well and then incubate for 1.5 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash buffer** (300 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 20 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µl of **HRP-conjugate** to each well. Mix well and then incubate for 1.5 hours at room temperature.
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 15-30 minutes at room temperature. Protect from light.
9. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining wash solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 20 second soak period following the addition of wash solution, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 minutes). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.