EIV22024E

ExCell Bio

ResiQuant® Ready-to-use CHO HCP ELISA Kit 2G

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	CRH00-3031S
	CRH00-3031
	CRH00-3032

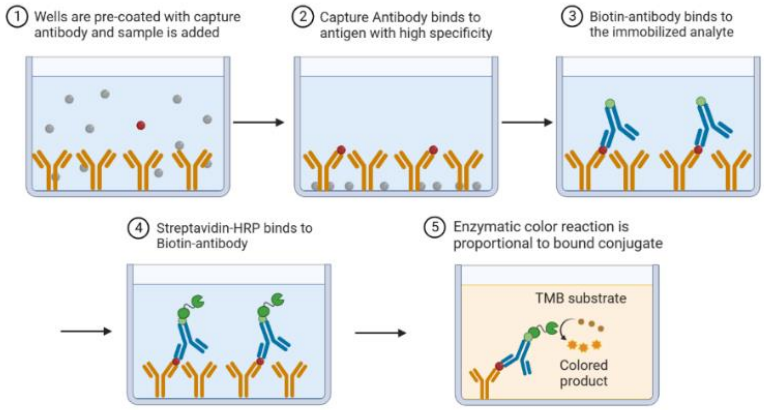
PRODUCT DESCRIPTION

The expression of therapeutic proteins in the CHO cells lines is economical and convenient for commercial drug production. However, the host cell proteins (HCPs) from CHO cells as a type of concomitant impurities are one of the major concerns in terms of drug safety. These impurities may diminish the efficacy of drug therapy and cause side effects or unexpected immune reactions. Therefore, control and monitor of the residual CHO HCP impurities is world widely required by all corresponding regulatory authorities. This product is a double-antibody sandwich ELISA kit. The antibodies were generated from rabbits and goats against the antigens harvested from the fermentation supernatant of CHO cells, cultured in the medium free of proteins. This kit can be used throughout the manufacturing process and for product release. For the first time, it is recommended to conduct a suitability study to confirm the sample matrix effect and determine the appropriate dilution conditions for sample testing.

PRINCIPLES OF THE TEST

The assay employs a sandwich immunoassay methodology for the quantitation of CHO HCP. Microplates have been coated with antibodies specific for CHO HCP. Subsequently, the standards or test samples are pipetted into the wells and CHO HCP present is bound by the immobilized antibody. After washing away unbound substances, Biotinylated anti-CHO HCP antibody is added to the wells and forms immune complex with standards or CHO HCP in the samples already bonded by immobilized antibody. After washing, horseradish peroxidase (HRP)-conjugated streptavidin is added and bound to biotinylated anti-CHO HCP antibody. Following a further round of washes to eliminate unbound Antibody-HRP conjugates, the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) is introduced, initiating an enzymatic reaction. This reaction involves HRP-mediated oxidation of TMB in the presence of hydrogen peroxide (H_2O_2), yielding a blue-colored product that exhibits maximum absorbance at 655 nm. The enzymatic reaction is subsequently halted by the addition of a stop solution, which turns the color to yellow with peak absorbance at 450 nm. The optical density (OD) value at 450 nm, measured by an enzyme-linked immunosorbent assay (ELISA) reader, is proportional to the concentration of CHO HCP within the samples. Quantitative determination of CHO HCP is achieved with a simultaneously prepared standard curve, using the standards supplied within the kit.

Schematic diagram:



PRODUCT PERFORMANCE

1. Sensitivity: The limit of detection (LOD) for CHO HCP is 0.5 ng/mL, and the low limit of quantitation (LLOQ) is 1.0 ng/mL.
2. Precision: The coefficient of variation (CV) within the quantitation range is no more than 20% for both intra and inter-assay.
3. Specificity: No cross-reactivity has been observed with *E.coli*, Vero, HEK293 whole cell lysates.

PRODUCT APPLICATION

This product is a generic assay kit designed for the quantitative detection of CHO HCP impurities in samples.

PRODUCT SPECIFICATIONS

Catalog Number	Name	Size
CRH00-3031S	ResiQuant® Ready-to-use CHO HCP ELISA Kit 2G	48T
CRH00-3031	ResiQuant® Ready-to-use CHO HCP ELISA Kit 2G	48T
CRH00-3032	ResiQuant® Ready-to-use CHO HCP ELISA Kit 2G	96T

REAGENTS PROVIDED

Name	96 Tests	48 Tests	Store
CHO HCP 2G Microplate	8× 12	8× 6	2℃ to 8℃
CHO HCP Standard Standards at 250, 100, 40, 16, 6.4, 2.6, 1 and 0 ng/mL, 1 mL/vial	8	8	2℃ to 8℃
CHO HCP 2G 100× Biotin-Antibody	60 µL	30 µL	2℃ to 8℃
100× Streptavidin-HRP	120 µL	60 µL	2℃ to 8℃
CHO HCP Assay Diluent	2× 25 mL	25 mL	2℃ to 8℃
20× Wash Buffer Concentrate	30 mL	30 mL	2℃ to 8℃
Substrate Solution	12 mL	6 mL	2℃ to 8℃ (Light-Sensitive)
Stop Solution	12 mL	12 mL	2℃ to 8℃
Plate Sealer	3	2	/

INSTRUCTION FOR USE

I. Materials required but not provided

1. Microplate reader capable of measuring absorbance at 450 nm (If wavelength correction is needed, additional wavelength at 630 nm or 570 nm is required).
2. 0.5-10 µL, 2-20 µL, 20-200 µL, 100-1000 µL adjustable micropipettes with disposable tips.
3. Microtiter plate rotator.
4. Deionized water.

II. Sample collection

1. Samples should be clarified and any precipitate should be removed by centrifugation.
2. The sample should be diluted appropriately according to the pre-determined condition. (It is recommended to complete the applicability study to confirm the appropriate dilution conditions for sample detection).

III. Preparation of reagents

1. It is recommended to remove the assay kit from the refrigerator 20 minutes in advance to allow it to equilibrate to room temperature.
2. Dilute 20× Wash Buffer Concentrate with deionized or distilled water to prepare the wash buffer. Store the unused solution at 2℃ to 8℃.
3. Biotinylated Antibody Working Solution: Freshly prepare every time the working solution by diluting CHO HCP 2G 100× Biotin-Antibody with CHO HCP Assay Diluent to 1× in an amount sufficient for the current

experiment.

4. Enzyme Conjugate Working Solution: Freshly prepare every time the working solution by diluting 100× HRP-Streptavidin with CHO HCP Assay Diluent to 1× in an amount sufficient for the current experiment.

IV. Washing

1. Automatic washing machine: set the volume to 300 µL, and the interval between aspiration and dispensing is 10 seconds. Wash the plate 5 times.

2. Manual plate washing: add 300 µL of wash buffer into each well, allow it to sit for 10 seconds, then shake off the liquid in the well. Invert the plate and blot it against clean paper towels. Wash the plate 5 times.

V. Assay procedure

1. Bring all reagents and samples to room temperature before use. Remove excess microplate strips from the plate frame, return them to the foil pouch containing a desiccant pack, reseal tightly and store at 2℃ to 8℃.

1. Set the blank well (if the plate is measured with dual-wavelength, the blank may not be necessary).

1. Prepare samples, standards and biotinylated antibody working solution in advance.

2. Add **100 µL** per well of either standards or samples. Seal the wells with the plate sealer. Incubate the plate at **37℃** and shake for **60 minutes**, using a microplate shaker (500 rpm).

3. Prepare biotinylated antibody working solution in advance.

4. Remove the liquid from the wells and wash the plate 5 times.

5. Add **50 µL** of biotinylated antibody working solution to each well. Seal the wells with the plate sealer. Incubate at **37℃** and shake for **60** minutes, using a microplate shaker (500 rpm).

6. Prepare enzyme conjugate working solution in advance.

7. Remove the liquid from the wells and wash the plate 5 times.

8. Add **100 µL** enzyme conjugate working solution to each well. Seal the reaction wells with the plate sealer. Incubate at **37℃** and shake for **60** minutes, using a microplate shaker (500 rpm).

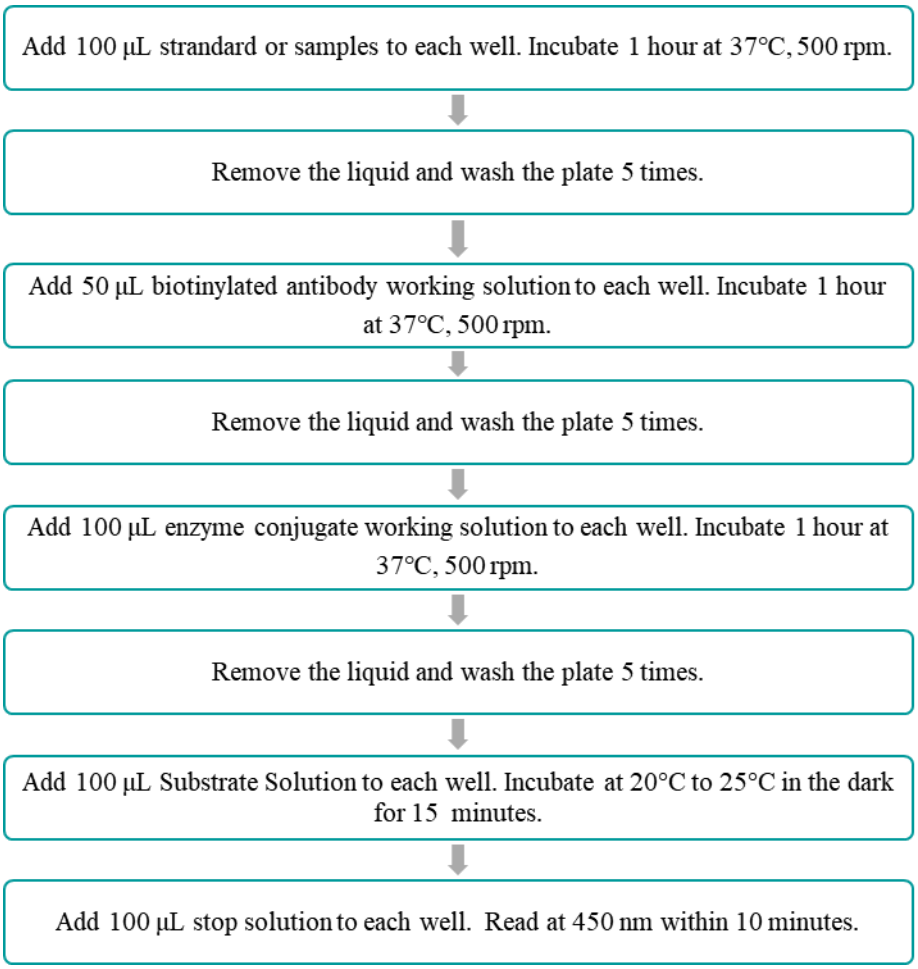
9. Remove the liquid from the wells and wash the plate 5 times.

10. Add **100 µL** of Substrate Solution to the wells, Incubate at **20℃ to 25℃** in the dark for **15** minutes.

11. Add **100 µL** of Stop Solution to the wells.

12. Read the optical density (OD) of each well within 10 minutes using a microplate reader set to 450 nm. If wavelength correction is available, the OD value at additional wavelength (630 nm or 570 nm) should be collected. For single wavelength mode, the OD value of blank well shall be subtracted from that of each Standard or sample.

VI. Assay Procedure Summary



ANALYSES OF RESULTS

1. Create a standard curve by plotting the mean optical density (OD) of each standard concentration as the y-axis against the standard concentration on the x-axis. It is recommended to use a four-parameter fitting equation for the standard curve. Calculate the concentration of samples based on their OD values.

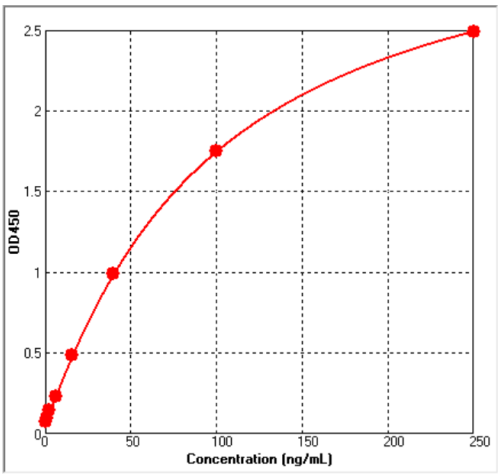
2. The coefficient of determination (R^2) for the standard curve should be no less than 0.99. Discard those obvious abnormal data, the deviation between the back calculated and the theoretical concentration of each standard should be within ±20%,(±25% for the upper or lower limits of quantitation).

3. The concentration of samples should be calculated with the standard curve in the parallel assay.

4. If the OD value of the sample exceeds the high end of the standard curve, the sample should be appropriately diluted before further measurement. Be aware that the dilution factor should be counted with back calculated concentration together.

Representative Standard Curve:

Standard (ng/mL)	**$OD_{450-630}$**			**Concentration (ng/mL)**		
	1	2	Average	1	2	Average
250	2.471	2.512	2.492	242.7	257.8	250.3
100	1.753	1.742	1.748	100.3	99.1	99.7
40	1.019	0.966	0.993	41.6	38.6	40.1
16	0.491	0.484	0.488	16.2	15.9	16.1
6.4	0.235	0.229	0.232	6.2	6.0	6.1
2.6	0.142	0.144	0.143	2.7	2.8	2.8
1.0	0.095	0.099	0.097	0.9	1.1	1.0
0	0.079	0.072	0.076	------	------	------
R^2	0.99998					



Note: This graph is provided for reference purpose only. Data analysis was using ELISA Calc software with a four-parameter fitting model.

NOTES

1. Store the reagents at 2℃ to 8℃. The reconstituted standard stock solution should be kept at 18℃ or below.
2. The concentrated Biotin-Antibody and Streptavidin-HRP are supplied in a small volume. Turbulence or inversion happened during transportation may cause spray of the solution elsewhere on the wall or the cap. Therefore, please spin the tubes shortly before use to harvest all liquid to the bottom of the tube.
3. In case crystal sediment appears in the 20× Wash Buffer Concentrate, warm it up at 37℃ to dissolve the sediment before use.
4. Do not mix or substitute reagents with those from other lots or different kits.
5. Ensure thoroughly mixing in preparing the solutions to ensure the reactions are consistent.
6. It is recommended to test all samples and standards in duplicate form at least.

DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.
2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.