Elabscience®

CHO HCP ELISA Kit

REF GEN001

TEST PRINCIPLE

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to CHO HCP. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for CHO HCP and Streptavidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain CHO HCP, biotinylated detection antibody and streptavidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of CHO HCP. You can calculate the concentration of CHO HCP in the samples by comparing the OD of the samples to the standard curve.

KIT COMPONENTS

Item	Specifications
ELISA Microtiter plate	96 wells
Standard substance	S1:200ng/mL, S2:100ng/mL, S3:30ng/mL, S4:10ng/mL, S5:3ng/mL, S6:1ng/mL, S7:0ng/mL
Biotinylated Detection Ab	12mL
HRP Conjugate	12mL
Concentrated Wash Buffer(20×)	50mL
Sample Diluent	50mL
Substrate Reagent	12 mL
Stop Solution	12 mL
Plate Sealer	3 pieces
Sealed Bag	1
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

OTHER SUPPLIES REQUIRED

Microplate reader with 450 nm wavelength filter
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃
Deionized or distilled water
Absorbent paper
Loading slot

STORAGE AND EXPIRY DATE

The unopened reagent kit is stored at 2-8℃ and has a shelf life of 12 months.

REAGENT PREPARARION

1. Bring all reagents to room temperature (18-25℃) before use. If the kit will not be used up in one assay, please only take out the necessary strips and reagents for present experiment, and store the remaining strips and reagents at required condition.
2. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.
3. Preparation of test sample: The test sample should be diluted to 3-10 mg/mL with sample diluent before test.
4. Preparation of quality control samples (optional): Dilute the test sample to 3-10 mg/mL (same as step 3), and then mix it with 30 ng/mL standard (Named as spiked quality control sample) or sample diluent (Named as the subtracted control sample) in a 1:1 ratio.

ASSAY PROCEDURE

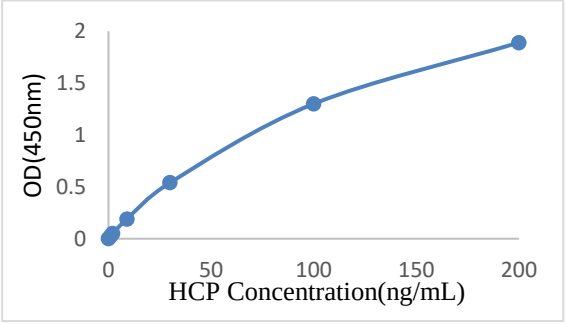
1. Determine wells for standard substance, control sample and sample. Add 100 µL each standard substance, control sample and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate or triplex.). Cover the plate with the sealer provided in the kit. Incubate for 90 min at room temperature.
   Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
2. Wash: After incubation, remove the plate sealer and aspirate the liquid of each well. Immediately add 300 µL of Wash Buffer to each well and wash. Repeat the washing procedure for 4 times with wash buffer and immerse for 30-60 sec each time.
3. Add 100 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 45 min at at room temperature.
4. Repeat step 3.
5. Add 100 µL of HRP conjugate to each well. Cover the plate with a new sealer. Incubate for 30 min at at room temperature.
6. Repeat step 3.
7. Add 100 µL of Substrate Reagent to each well. Cover the plate with sealer. Incubate for 10 min at at room temperature. Avoid bubbles.
8. Add 100 µL of Stop Solution to each well. Avoid bubbles.
9. Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm within 20 min. Fit the data with four parameters. The concentration range of the standard curve is 1-200 ng/mL. It is recommended to use the absorption value of 0 ng/mL standard as a blank control for the suitability of the system.

NOTES

1. If the HCP content of the sample to be tested exceeds 200 ng/mL, the dilution ratio should be expanded.
2. When measuring the sample for the first time, it is recommended to determine the minimum required dilution (MRD) through gradient dilution.
3. Try to avoid using edge holes, so as not to affect the stability of the experimental data.
4. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
5. A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
6. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab and other stock solutions should be stored according to the storage conditions in the above table.
7. The microplate reader should be able to be installed with a filter that can detect the wave length at 450 ± 2 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
8. Do not mix or substitute reagents with those from other lots or sources.
9. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
10. The kit should not be used beyond the expiration date on the kit label.

RESULT ANALYSIS

1. Average the duplicate or triplex readings for each standard and samples. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.
2. Calculation of recovery rate for quality control samples: (HCPs concentration of spiked quality control samples minus HCPs concentration of subtracted control sample)/15 × 100%. The recovery rate of quality control samples should be within the range of 50% - 150%. If it is not within the range, it is necessary to further investigate whether the concentration of the sample to be tested is lower than MRD and the interference of the sample matrix.



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