Vero Cell HCP Fast ELISA Catalog Number: TBS31005

For the quantitative determination of Vero HCP residues in biological samples.

INTRODUCTION

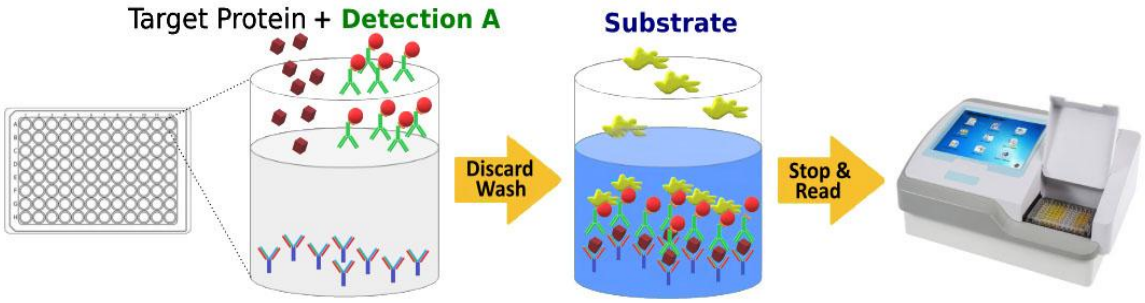
Host Cell Proteins (HCPs) are process-related impurities generated by the host organisms in biopharmaceutical manufacturing. They must be strictly monitored and removed during purification to prevent adverse patients immune reactions and drug instability. ELISA is the standard screening method for residue HCP determination. The Vero cell HCP ELISA is used to detect and quantify Vero cell HCP residues in biotherapeutics to ensure the quality and safety of biopharmaceutical therapeutics.

The Tribioscience Vero cell HCP Fast ELISA is designed to quantitatively detect Vero cell HCP residues in different biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay flexible, accurate, simple, and time saving. The measurement can be finished in 2 hours, not needing 4-5 hours (Fig. 1). The detection range is from 1 to 200 ng/mL. The levels of Vero HCP samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for Vero host cell protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (Fig. 1). An antibody specific to Vero HCP is pre-coated onto a microplate. Standards or samples and detection antibody are pipetted into the wells and concurrently incubated to form a sandwich complex in one step. Simply aspirate each well, following a wash, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is proportional to the amount of Vero HCP bound in the initial step. The intensity of the color is measured by plate reading at 450 nm.

Fig. 1: Assay Principle:



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Vero HCP Microplate	TBS31005A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific to Vero HCP.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Vero HCP Standard	TBS31005B	90 µL of Vero HCP protein (2 µg/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS31005C	55 µL of HRP Vero HCP antibody.	May be stored for up to 3 months at 2-8 °C.*
Assay Diluent	TBS31005D	25 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	15 mL of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 mL of 2 N sulfuric acid.	

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

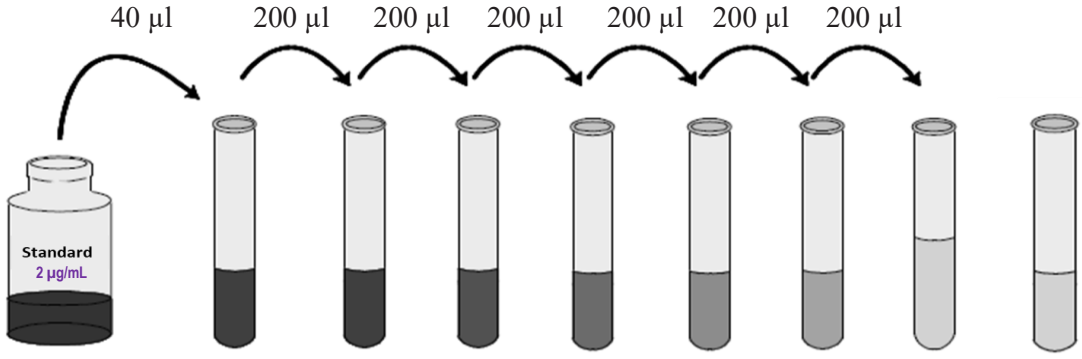
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).

Detection A working solution preparation: Add 50 µL of **Detection A Stock** (HRP-Vero HCP) to 1950 µL Assay Diluent to prepare Detrection A working solution. Add 20 µL to each well.

Vero HCP Standard Preparation: Label test tubes as #1 through #8. Pipet 360 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #7 **as diagram below (Fig.2).**

1. Add 40 µL of the Vero HCP Standard stock solution (2 µg/mL) by dilution of 10X to tube #1 (200 ng/mL), and mix.
2. Make 2x serial dilutions of the standard using the 200 ng/mL standard solution (tube #1) from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly and gently before the next transfer. The standard concentration in tubes 1 through 7 will be 200, 100, 50, 25, 12.5, 6.25, and 3.13 ng/mL. Tube# 8 is Standard 0 **as blank**.

Fig.2 Diagram for Vero HCP standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	360	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	40	200	200	200	200	200	200	0
Final Conc (ng/mL)	200	100	50	25	12.5	6.25	3.13	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, or sample, or control per well in duplicate manner.
2. Add 20 µL of Detection A working solution to the above standard and sample or control of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at RT for 2 hours gently shaking.
3. Aspirate each well and wash 4 times by filling each well with 200 µL of Wash Buffer and sitting for 1 minute for each wash (Complete removal of liquid at each step is essential to good performance). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of TMB Substrate to each well. Incubate at RT for 10-20 min (*Protect from light*) for color development. The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density (OD) of each well within 10-20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract

readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will be correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard OD.

Create a standard curve 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. The data may be linearized by plotting the log of the Vero HCP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

LOD is about 1.1 ng/mL
LOQ is 4.9 ng/mL
The Intra-assay CV is 3.79% and the Inter-assay CV is <10%.

SPECIFICITY

This assay recognizes Vero HCP.

RELATIVE PRODUCTS

CHO HCP ELISA (TBS31004)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)

For research use only.

Fig. 3 Vero HCP Standard Curve