H^5 Host Cell Protein (HCPs) ELISA Kit

96 Tests Kit
Enzyme Immunoassay for the Quantitative Detection of H^5 Host cell proteins (HCPs)
For Research and Manufacturing only
Catalogue No. JTC233-H^5

Introduction

H^5 cell (BTI-Tn-5B1-4) is a clone isolated from ovary cells of female Trichoplusia ni insect, which is used to express recombinant proteins using Baculovirus expression vector system. This host cell is adapted to grow in serum-free medium. Ten-fold higher recombinant protein expression in H^5 cells compared to SF9 cells has attracted the attention of pharmaceutical companies to produce recombinant protein products in this cell line. In this context, Cervarix, a FDA approved HPV vaccine, has been manufactured in Trichoplusia ni insect cell line. The products manufactured in this system have the potential of impurity with host cell proteins (HCPs). These impurities can affect the efficacy of the vaccine and lead to adverse toxic or allergic reactions. Therefore, it is desirable to minimize HCP impurities in the recombinant biopharmaceutical products. Therefore, JTC H^5 HCP ELISA kit is designed to quantitative measure of HCPs contamination in pharmaceutical products manufactured in H^5 cell expression systems.

Test principle

JTC H^5 HCP Kit is based on the principle of a sandwich solid phase ELISA. The assay system utilizes an anti-H^5 HCP polyclonal antibody for solid phase immobilization (on the wells) and an anti-H^5 HCP polyclonal antibody for the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, after incubation and washing, the enzyme conjugate will be added, resulting in the sandwiched formation of H^5 HCP between solid phase and conjugated antibodies. After second wash step a solution of 3,3′,5,5′-Tetramethylbenzidine (TMB) is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped after adding of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. A 4-parameter logistic (4-PL) fit standard curve is used to calculate the concentration of HCPs in the sample. The concentration of H^5 HCP is proportional to the color intensity of the test sample in comparison with the standards.

Materials provided with the kit

1. Antibody coated wells (1×96-well plate), microtiter wells coated with polyclonal anti- H^5 HCP antibody
2. Enzyme conjugate (1 vial, 12 mL), polyclonal anti-H^5 HCP antibody labeled with HRP in buffer, ready to use
3. Standards set (1 mL/vial), containing 0, 3, 6, 12, 25, 50 and 100 ng/mL of H^5 HCP, ready to use
4. Chromogen substrate reagent (1 vial, 12 mL), benzidine and hydrogen peroxide, ready to use solution
5. Wash solution (1 vial, 50 mL), contain Phosphate Buffered Saline solution with 0.05 % Tween 20 as detergent, concentrated (20×)
6. Stop solution (1 vial, 12 mL), 1 molar hydrochloric acid solution
7. Cardboard sealer (1 pcs)
8. Brochure Barcode

Materials required but not provided

- ELISA reader with 450 nm filter (if possible 630 nm as reference filter)
- Orbital rotator with a minimum speed of 200 rpm
- Precision pipette: 100 µL
- Disposable pipette tips
- Distilled water
- Absorbent paper

Notes for consumers

1. The contents of the kit should be used only for the current kit.
2. All contents of the kit are for research use only.
3. Kit should be used only by qualified technicians.
4. Kit is designed and manufactured only for the measurement of H^5 HCP in biopharmaceutical products.
5. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
6. Kit is for Research or Manufacture Use Only.

Storage and stabilities

1. Kit should be stored at 2-8 °C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. For long term storage, in concentrated washing solution, crystals may form. Before preparing the work wash solution, place the vial at 37 °C to dissolve the crystals. Dilute the concentrated washing solution with distilled water, 1/20.

General information

1. Be sure to bring all reagents at room temperature 30 minutes before starting the test.
2. All steps should be performed non-stop from the start of the test. Do not allow the wells to dry between incubation stages.
3. Use a disposable sampler tip for each sample.
4. Strips should be read within 30 minutes after adding stop solution since color will fade over time.
5. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
6. Precision on duplicate samples should yield coefficients of variation (CV) of less than 10% for samples. In cases, where the CV of two replicates of each sample is high, the plate washing process should be evaluated.
7. One of the most important factors in achieving the accurate result is the appropriate incubation time. Before starting the test, prepare the required materials and solutions, this will be improved the accuracy by decreasing the time interval between the sampling steps.
8. In samples with high concentrations of HCP, the linearity test is not observed in diluted samples. In these conditions, the minimum required dilution (MRD) must be calculated for the final or in-process production samples. A very important point is to use a compatible diluent to achieve accurate recovery.

Reagents preparation

1. Bring all reagents to reach room temperature (20-25 °C) before use.
2. Working wash solution: Dilute concentrated wash solution 1:20 with distilled water before use and store working wash solution at 2-8 °C.

Test procedure

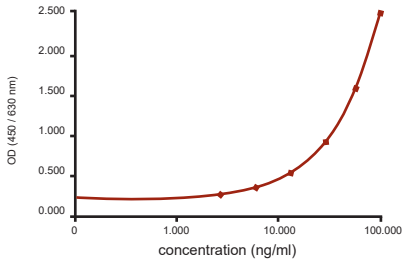
1. Choose the appropriate number of coated wells and keep remaining wells in tightly closed special bag.
2. Dispense 100 µL of standards and samples into the wells in duplicate and shake wells gently for 15 seconds to mix well.
3. Cover the microtiter wells with cardboard sealer firmly. Incubate plate on orbital shaker at 200-600 rpm for 90 minutes at room temperature (20-25 °C).
4. Remove the sealer and take out wells contents by flicking the microplate into a waste container.
Rinse and flick the microtiter wells 5 times (each time with 300 µL of working wash solution). Strike the wells gently onto absorbent paper or paper towels to remove all residual droplets.
5. Add 100 µL of anti-H^5-HRP conjugate into the wells.
6. Seal the plate with cardboard sealer again. Incubate plate on orbital shaker at 200-600 rpm for 30 minutes at room temperature (20-25 °C).
7. Repeat step 4.
8. Dispense 100 µL of chromogen/substrate (TMB) into the microplate wells.
9. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
10. Stop the reaction by adding 100 µL of stop solution to the microplate wells.
11. Measure absorbance at 450 nm by ELISA reader (use 630 nm filter as reference filter if it is available).

Result calculation

1. Calculate mean absorbance value of standards and samples at 450 nm (use 630 nm filter as reference filter if it is available).
2. Construct a 4-PL fit standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL using curve fitting software, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Calculate the corresponding concentration of H5 HCP in the sample (ng/mL) from the standard curve using the mean absorbance value for each sample.
4. mean absorbance value for each sample.

Example of H5 HCP standard curve

Standards (ng/mL)	OD (450/630 nm)	Mean OD
0	0.17	0.16
	0.15	
3	0.31	0.32
	0.33	
6	0.49	0.48
	0.48	
12	0.73	0.75
	0.78	
25	1.20	1.19
	1.18	
50	1.66	1.67
	1.68	
100	2.44	2.42
	2.40	



Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Limitations of the measurement method

1. Before using the kit to measure H5 HCP, the quality of antibodies used in this kit and the test method should be evaluated by each laboratory in terms of specificity, accuracy and precision.
2. Despite designing the kit to avoid interfering with different agents, very excessive concentrations of some samples, can also additionally interfere in the accurate measurement of HCPs. Drug samples were evaluated in 1 mg/mL concentration of the drug to determine the performance characteristics of the kit.

Performance characteristics

1. Sensitivity

The lower limit of detection (LOD) is defined as that concentration corresponding to a signal three standard deviations above the mean of the zero standard. LOD is ~0.8 ng/mL.
The limit of quantitation (LOQ), is the lowest concentration at which CV is less than 20% with acceptable accuracy. The LOQ is ~3 ng/mL.

2. Test precision

Both intra-(n=20 replicates) and inter-(n=20 replicats) assays precision was determined on three drug substance samples with different concentrations of H5 HCP. Results are shown in Tables 1 and 2:

Table 1. Intra-assay

Sample	No. of tests* performed	Means (ng/mL)	SD (ng/mL)	CV (٪)
1	20	10.56	0.36	3.4
2	20	39.97	1.38	3.45
3	20	92.23	9.02	9.78

Table 2. Inter-assay

Sample	No. of tests* performed	Means (ng/mL)	SD (ng/mL)	CV (٪)
1	20	10.74	0.82	7.64
2	20	40.66	2.23	5.48
3	20	82.34	7.85	9.53

***Each test has been run in duplicate.**

3. Specificity

The H5 HCP ELISA kit can specifically measure the amount of HCP of the H5 host cell and does not interfere with the proteins of other expression hosts, including E. coli and CHO.
It can also detect H5 HCP with different concentrations of HCP in the drug samples in different stages of production.

4. Matrix interference

By spiking H5 HCP antigen with a specified concentration of 30 ng/mL in the pharmaceutical sample containing known amount of H5 HCP, interference related to other proteins was not observed. Also, by spiking H5 HCP antigen in the samples containing different concentrations of BSA from 1 to 8 mg, matrix interference was not observed. The recovery test results were in the range of 80-120%.

5. Hook effect

To rule out possible hook effect occurrence, the H5 HCP assay was done on samples with high concentration of H5 HCP (up to 750 µg/mL) and no “hook effect” was seen.

Schematic Procedure of H5 HCP test		
Reagent	Standard	Sample
Standard	100 µL	-
Sample	-	100 µL
Shake the plate gently for 15 seconds and then cover the wells with cardboard sealer. Incubate for 90 minutes on the rotator at 200 rpm at RT. Remove the cardboard sealer of the plate and empty the contents. Wash the wells 5 times according to the washing instructions.		
Anti- H5 HCP-HRP conjugate	100 µL	100 µL
Cover the wells with the cardboard sealer. Incubate for 30 minutes on a rotator at 200-600 rpm at RT. Remove the cardboard sealer from the plate and empty the contents of the wells. Wash the wells 5 times according to the washing instructions.		
TMB	100 µL	100 µL
Incubate wells for 15 minutes at RT in dark.		
Stop	100 µL	100 µL
Read absorbance at 450 nm (use 630 nm as reference filter if it is available).		

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