KRIBIOLISA™ High Five (Hi5) HCP ELISA

REF : KBBP21

Ver 1.1

RUO

Enzyme Immunoassay for the Quantitative Determination of HI5 Host Cell Proteins in cell culture supernatant and biological solutions

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBBP21 Σ **96 tests**

KRISHGEN BioSystems For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgenbio.com

Introduction:

High Five cells (sometimes referred to as High-Five or Hi5) represent a safe, effective and inexpensive platform for protein production. Indeed, their remarkable ability to produce very large amounts of recombinant proteins – such as diagnostic reagents and recombinant vaccines – remains unmatched. The High Five cells were originally isolated from insects in BTI's Granados lab in the late 1980's.

The baculovirus expression vector system (BEVS) in insect cells offers highly scalable and inexpensive protein production. High Five cells and sub-clones have been reported to produce 2-10x high levels of recombinant proteins compared to Sf9/Sf21 insect cells.

BTI is the sole proprietor of High Five cells and related sub-clones. (BTI; Boyce Thomson Institute, NY, USA).

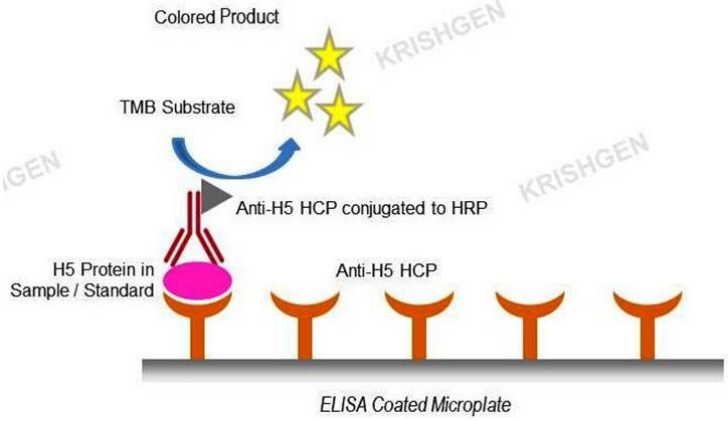
The KRIBIOLISA Hi5 (High Five) HCP ELISA kit is designed to quantitatively measure HCPs contamination in pharmaceutical products manufactured using the High Five Cells expression systems.

Intended Use:

This generic kit is intended in determining the presence of High Five cells Host Cell Proteins contamination in various products that are manufactured through recombinant expression in Hi5 cells. The kit has been validated successfully for testing of in process and final product HCPs in variety of products regardless of growth and purification process.

Principle:

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Hi5 HCP present in the sample are bound by the antibodies. HRP conjugated antibody is added and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Hi5 HCP in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



Materials Provided:

1. Hi5 HCP Antibody Coated Microtiter Plate (8 x 12 wells) - 1 no
2. Hi5 HCP Standard (1ml / vial) – 0, 3, 6, 12, 25, 50 and 100 ng/ml
3. Anti-Hi5 HCP:HRP Conjugate - 12 ml
4. (20X) Wash Buffer - 25 ml
5. TMB Substrate - 12 ml
6. Stop Solution - 12 ml
7. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.
7. 37°C incubator
8. Timer.

Handling/Storage:

1. All reagents should be stored as indicated on the component label.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Sample Preparation and Storage:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. **Cell Culture Supernatant-** Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
2. **Cell Culture Lysate**: Commercial RIPA kits are recommended to follow the instructions provided. Generally 0.5 ml RIPA lysis buffer would be appropriate to $2x10^6$ cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
3. **Other Biological Fluids**: Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at - 20°C (assay ≤ 1 month) or -80°C (assay ≤ 2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make **Wash Buffer (1X);** dilute **25 ml of (20X) Wash Buffer in 475 ml of DI water**.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of Hi5 HCP. High Dose Hook Effect is due to excess of antibody for very high concentrations of HI5 HCP present in the sample.

3. Hi5 HCP concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
4. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Hi5 HCP.
5. It is recommended that all Standards and Samples be assayed in duplicates or triplicates.
6. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
7. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
8. The plates should be read within 30 minutes after adding the Stop Solution.
9. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.

2. Add **100 ul Standards and Samples** to respective wells.

3. Cover the plate with a sealer and incubate for 90 minutes at room temperature on a shaker at 200-600rpm.

4. Aspirate and wash plate 4 times with diluted Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

5. Pipette **100 ul Anti-Hi5 HCP:HRP Conjugate** to all wells. Mix well.

6. Cover the plate with a sealer and incubate for 30 minutes at room temperature on a shaker at 200-600rpm.

7. Aspirate and wash as per Step (4) above.

8. Pipette **100 ul TMB Substrate** in all the wells.

9. Incubate the plate at **room temperature** for **15 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette **100 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards and Samples after subtracting the zero standard (blank) absorbance values. Using Graph Paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown HI5 HCP concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the HI5 HCP Concentration.

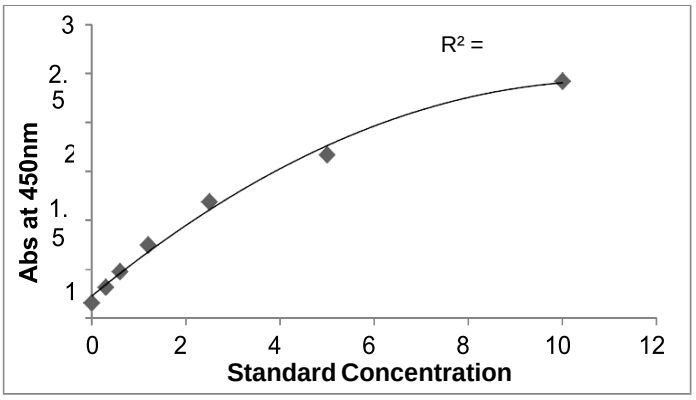
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL (2^{nd} order) is best recommended for automated results.

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

Typical Data

Standard Concentration (ng/ml)	Abs1	Abs2	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration	STD Deviation	%CV	Net Signal Difference
0	0.17	0.15	0.16	0.13	---	---	---	---
3	0.31	0.33	0.32	2.56	85.39	0.31	11.14	0.16
6	0.49	0.48	0.48	5.73	95.54	0.19	3.23	0.32
12	0.73	0.78	0.75	12.32	102.67	0.23	1.86	0.59
25	1.20	1.18	1.19	26.46	105.85	1.04	4.02	1.03
50	1.66	1.68	1.67	47.87	95.74	1.51	3.08	1.51
100	2.4	2.44	2.42	101.03	101.03	0.73	0.72	2.26

Typical Graph



Abs = absorbance at 450nm

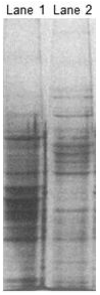
Quality Control:
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Standard Calibration Range:
3 ng/ml - 100 ng/ml

Sensitivity:
Limit of Quantification: It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 0.188 ng/ml.
Limit of quantification (LOQ): It is defined as the lowest concentration at which CV is less than 20% with acceptable accuracy and the LOQ is 3 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of Hi5 HCP. No significant cross-reactivity or interference between HI5 HCP and analogues was observed. The antigen used was developed from mock fermented Hi5 media. The western blot was done to view the coverage of the HCP proteins. (picture below).



The antibodies developed against the purified antigen are rabbit polyclonals affinity purified.

Precision

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (3 ng/ml) and high (100 ng/ml) concentrations. While actual precision may vary from laboratory to and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results:

Pool	Intra Assay %CV	Inter Assay %CV
Low	~9.78%	~9.53%
High	~3.45%	~7.64%

Dilutional Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Hi5 HCP and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Cell Culture Supernatant (n=10)	91-105%	92-106%	90-111%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Interpolated Concentration
1A 2A	Standard No.1 Standard No.1			
1B 2B	Standard No.2 Standard No.2			
1C 2C	Standard No.3 Standard No.3			
1D 2D	Standard No.4 Standard No.4			
1E 2E	Standard No.5 Standard No.5			
1F 2F	Standard No.6 Standard No.6			
1G 2G	Standard No.7 Standard No.7			
1H 2H	Sample Sample			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

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SYMBOLS KEY

MTP	**Coated Microtiter Plate (8x12 wells)**
STD	**Standard**
HRP CONJ	**Conjugate Horseradish Peroxidase**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**