KRIBIOLISA® CHO (Wide Coverage) HCP ELISA

REF : KBBP12200

Ver SK1-0

RUO

Enzyme Immunoassay for the Quantitative Determination of CHO HCP in Biological preparations

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 KBBP12200 Σ 96 tests

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Introduction:

The KRIBIOLISA® ELISA kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma and cell culture supernatant as validated with the kit. The kit employs a sandwich ELISA technique which leads to a higher specificity and increased sensitivity compared to conventional competitive ELISA kits which employ only one antibody. Double antibodies are used in this kit.

Intended Use:

The KRIBIOLISA® CHO (Wide Coverage) HCP ELISA kit is intended for use in determining the presence of host cell proteins (HCPs) in products manufactured by expression in CHO host cells such as monoclonal antibodies, recombinant proteins and vaccines etc.

Principle:

The method employs sandwich ELISA technique. Polyclonal antibodies are pre-coated onto microwells. Samples, standards, Anti-CHO HCP HRP conjugate are pipetted into microwells and incubated to form an immune 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 CHO HCP in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti-CHO HCP Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. CHO HCP Standard (lyophilized, concentrated, 0.72 ug) - 2 vials
3. Anti-CHO HCP:HRP Conjugate (concentrated) - 120 ul
4. Standard Diluent – 1.5 ml
5. Assay Diluent - 2 x 25 ml
6. (20X) Wash Buffer – 2 x 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 6 ml
9. 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:

Test Samples: Cell culture fluid, In-process samples, drug substances and drug product. Samples should be clear and transparent and insoluble substances need to be removed from samples though centrifugation or filteration.

Test Sample Preparation: The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided assay diluent, and several trials may be necessary. The test sample must be well mixed with the assay diluent.

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**.
4. **Anti-CHO HCP:HRP Conjugate Working Solution**: Dilute the Anti-CHO HCP:HRP Conjugate with Assay Diluent at 1:100 and mix them thoroughly (i.e. Add 1 ul of Anti-CHO HCP:HRP Conjugate into 99 ul of Assay Diluent).
6. **Standards Preparation**: Reconstitute original CHO HCP Standard with 0.5 ml of standard Diluent. Keep the standard for 15 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the below table.

Standard Concentration	Standard Vial	Dilution Particulars
0.72 ug	Lyophilized Standard	Reconstitute with 0.5 ml standard Diluent
128 ng/ml	Standard No.7	88.9 ul Reconstituted Standard + 911.1 ul Assay Diluent
64 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Assay Diluent
16 ng/ml	Standard No.5	250 ul Standard No.6 + 750 ul Assay Diluent
4 ng/ml	Standard No.4	250 ul Standard No.5 + 750 ul Assay Diluent
2 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Assay Diluent
1 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Assay Diluent
0 ng/ml	Standard No.1	500 ul Assay Diluent

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 CHO HCP. High Dose Hook Effect is due to excess of antibody for very high concentrations of CHO HCP present in the sample.
3. CHO 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 CHO 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 prepared Standards and Samples** to respective wells.

3. Add **100 ul Anti-CHO HCP:HRP Conjugate Working Solution** to all wells. Mix well.

4. Cover the plate with a sealer and incubate for 180 minutes at room temperature on a shaker at 500-600rpm.

5. 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.

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

7. 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.

8. Pipette **50 ul** of **Stop Solution** to all wells. The wells should turn from blue to yellow in color.

9. 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. 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 CHO 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 CHO HCP concentration.

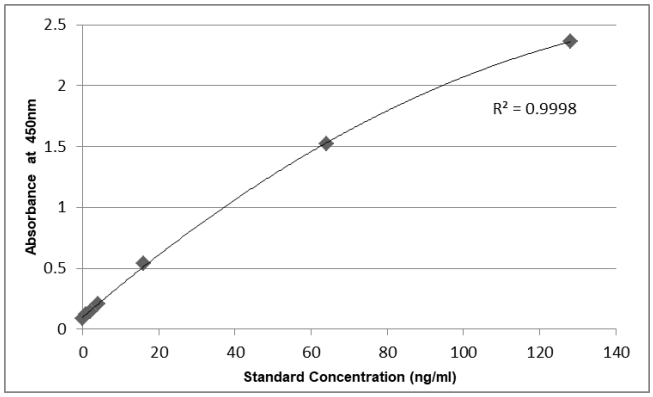
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

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

Concentration ng/ml	Abs1	Abs2	Abs3	Mean Abs	Interpolated concentration ng/ml	% Recovery
0	0.091	0.086	0.089	0.089	-	-
1	0.122	0.119	0.12	0.121	1.06	94.00
2	0.154	0.149	0.141	0.148	1.94	103.13
4	0.207	0.211	0.198	0.205	3.84	104.26
16	0.554	0.548	0.524	0.542	16.15	99.10
64	1.591	1.509	1.457	1.519	63.92	100.13
128	2.456	2.395	2.239	2.363	128.04	99.97

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:
1 ng/ml – 128 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 1 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of CHO HCP. No significant cross-reactivity or interference with MDCK, Vero, HEK293T, P.pastoris and SF9 cells.

Precision:
Both intra-assay and inter-assay precision were evaluated on 3 levels of concentration – Low (3 ng/ml), Medium (50 ng/ml), and high (100 ng/ml).

Particulars	Low	Medium	High
Intra-Assay	7.8	6.5	8.6
Inter-Assay	10.6	11.6	7.8

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 (12 x 8 wells)**
STD	**Standard**
HRP CONJ	**Conjugate Horseradish Peroxidase**
STD DIL	**Standard Diluent**
ASY DIL	**Assay Diluent**
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**