KRIBIOLISA® Pichia Pastoris (Wide Coverage) HCP ELISA

REF : KBBP02

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of Pichia Pastoris (Wide Coverage) HCP originated from Pichia Pastoris cells

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 KBBP02 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® Pichia Pastoris (Wide Coverage) HCP ELISA kit is intended for use in determining the presence of host cell proteins contamination in products manufactured with Pichia Pastoris host cells, such as vaccines, influenza virus.

Principle:

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

Materials Provided:

1. Pichia Pastoris HCP Antibody Coated Microtiter Plate (12 x 8 wells) - 1 no
2. Pichia Pastoris HCP Standard (lyophilized, concentration indicated on the vial) - 2 vials
3. Anti-Pichia Pastoris HCP:HRP Conjugate (concentrated) - 120 ul
4. Standard Diluent – 1.5 ml
5. Assay Diluent - 2 x 25 ml
6. (10X) Wash Buffer – 2 x 25 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 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: In-process, harvested bulk, drug substance and Drug Product Make sure that the samples are clear and transparent, and insoluble substances need to be removed by 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 (10X) Wash Buffer in 225 ml of DI water**.
4. **Anti-Pichia Pastoris HCP:HRP Conjugate Working Solution**: Dilute the Anti-Pichia Pastoris HCP:HRP Conjugate with Assay Diluent at 1:100 and mix them thoroughly (i.e. Add 1 ul of Anti-Pichia Pastoris HCP:HRP Conjugate into 99 ul of Assay Diluent).
6. **Standards Preparation**: Reconstitute original Pichia Pastoris HCP Standard with 0.5 ml of Standard Diluent to get a concentration per ml as indicated on the vial label*. 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.

   *Concentration on reconstitution is indicated on the vial label. Upon reconstitution, dilute with the assay diluent to the highest concentration 200 ng/ml and then subsequently follow the dilution table below for preparation of the other standards.

Standard Concentration	Standard Vial	Dilution Particulars
0.5ml*	Lyophilized Standard	Reconstitute with 0.5 ml Standard Diluent
Dilute appropriately using the Assay Diluent to make the first concentration of 200 ng/ml.		
200 ng/ml	Standard No.7	Reconstituted Standard + Assay Diluent
100 ng/ml	Standard No.6	300 ul Standard No.7 + 300 ul Assay Diluent
50 ng/ml	Standard No.5	300 ul Standard No.6 + 300 ul Assay Diluent
20 ng/ml	Standard No.4	120 ul Standard No.6 + 480 ul Assay Diluent
10 ng/ml	Standard No.3	120 ul Standard No.5 + 480 ul Assay Diluent
2 ng/ml	Standard No.2	120 ul Standard No.3 + 480 ul Assay Diluent
0 ng/ml	Standard No.1	Only 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 Pichia Pastoris (Wide Coverage) HCP. High Dose Hook Effect is due to excess of antibody for very high concentrations of Pichia Pastoris (Wide Coverage) HCP present in the sample.
3. Pichia Pastoris (Wide Coverage) 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 Pichia Pastoris (Wide Coverage) 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-Pichia Pastoris 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 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 **20 minutes**. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

8. Pipette **100 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 Pichia Pastoris (Wide Coverage) 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 Pichia Pastoris (Wide Coverage) HCP concentration.

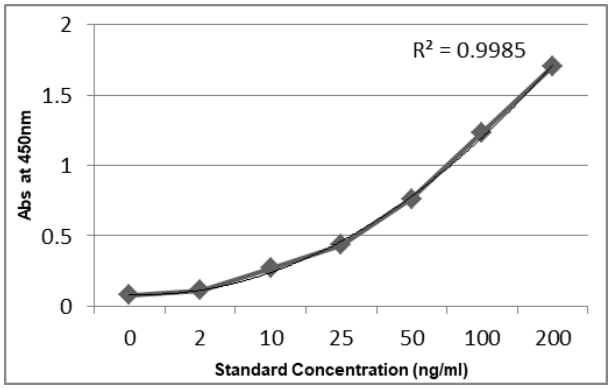
If samples were diluted, multiply by the appropriate dilution factor. Software which is able to generate a cubic spline curve-fit or 4-PL is best recommended for automated results.

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	Abs 1	Abs 2	Abs 3	Mean Abs	Interpolated Concentration ng/ml	% Recovery
0	0.079	0.078	0.076	0.078	0	-
2	0.117	0.111	0.115	0.114	1.89	94.4
10	0.271	0.27	0.27	0.270	12.27	122.7
25	0.434	0.444	0.423	0.434	23.41	93.6
50	0.763	0.768	0.753	0.761	49.17	98.3
100	1.244	1.225	1.229	1.233	102.27	102.3
200	1.748	1.681	1.684	1.704	198.47	99.2

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:
2 ng/ml – 200 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 2 ng/ml.

Specificity:
This assay has high sensitivity and excellent specificity for detection of Pichia Pastoris (Wide Coverage) HCP. No significant cross-reactivity or interference with E.coli, CHO and Sf9 cells, cross-reactivity less than 1% with Saccharomyces cerevisiae and Hansenula polymorpha.

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 (2 ng/ml) and high (200 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	~12%	~10%
High	~10%	~10%

Dilutional Linearity
The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Pichia Pastoris 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 (12 x 8 wells)
STD	Standard
HRP CONJ	Conjugate Horseradish Peroxidase
STD DIL	Standard Diluent
ASY DIL	Assay Diluent
10X WASH BUF	(10X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature