KRIBIOLISA™ *Pichia pastoris* Host Cell Proteins ELISA

REF: KBBP02

Ver 4.0

RUO

Immunoassay for quantitative determination of P. pastoris Host Cell Proteins

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:

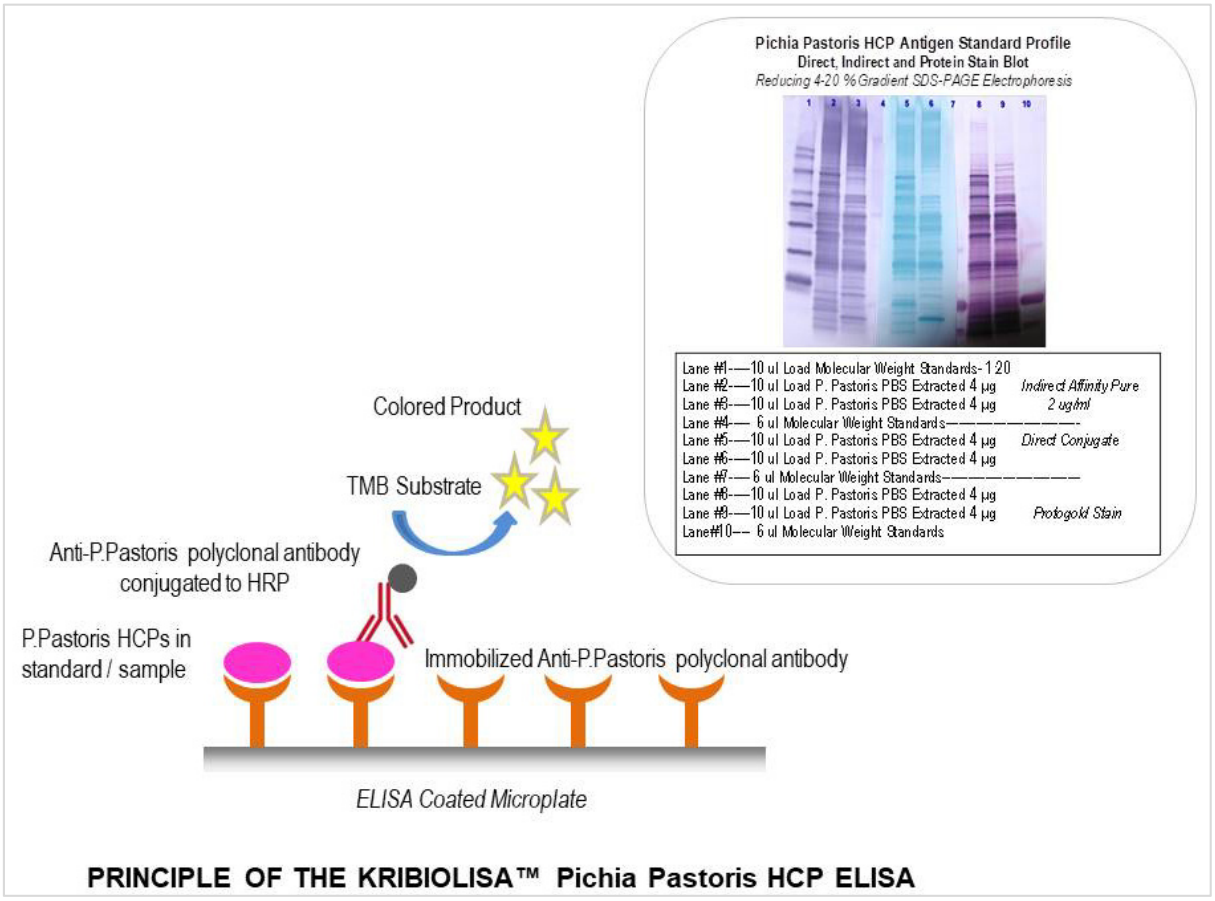
A variety of proteins and pDNA which are used as therapeutic agents in humans and animals are produced through recombinant expression in *P.pastoris.* The manufacturing and purification process of these products tends to leave the potential for contamination by Host Cell Proteins (HCPs) from P.pastoris which may result in adverse toxic or immunological reactions that ultimately affect the efficacy of the therapeutic agent. The simple, objective and semi quantitative ELISA is a highly sensitive method that aids in purification process development, process control, quality control and product release testing optimally.

Intended Use:

This KRIBIOLISA Pichia Pastoris HCP ELISA is a generic kit intended in determining the presence of *P. pastoris* Host Cell Proteins contamination in various biotechnology products that are manufactured through recombinant expression in *P. pastoris*. 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:

This assay is based on the Sandwich ELISA procedure. Samples containing *P.pastoris HCPs* are reacted with already coated affinity purified capture anti-*P.pastoris* antibody and bind to them. Plates are washed with wash buffer to remove unbound reactants. Anti *P.pastoris*-HRP conjugate is added which results in the formation of a sandwich complex of solid phase antibody-HCP-enzyme labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure. The substrate 3, 3',5, 5' Tetra Methyl Benzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of *P. pastoris* HCPs present.



PRINCIPLE OF THE KRIBIOLISA™ Pichia Pastoris HCP ELISA

Materials Provided:

Part	Description	Qty
Anti-*P.pastoris* Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with Anti-*P.pastoris* antibody.	1 x 96 wells
P.pastoris HCP Standards (0.5ml/vial)	Recombinant *P.pastoris* HCP Standard, (0, 15.6, 31.3, 62.5,125, 250, 500, 1000 ng/ml)	8 x 0.5ml
Anti-*P.pastoris* HRP Conjugate	Buffered protein base with preservative thiomersol <0.01%	12 ml
(5X) Sample Diluent	Buffered protein base with preservative thiomersol <0.01%	12 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time.	25 ml
TMB Substrate	Stabilized Chromogen	12 ml
Stop Solution	2N Sulfuric Acid	12 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at 2-8°C.
2. All the reagents and wash solution are stable until the expiration date of the kit.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

Specimen Collection and Handling:

Specimens should be clear. Samples should be run at a number of dilutions to ensure accurate quantitation. Incase additional sample diluent is required, please contact us at +91-22-49198700 | email: sales@krishgen.com to order.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.

2. If the *P.pastoris* HCP concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect. High Dose Hook Effect may be observed in samples with very high concentrations of *P.pastoris* HCP, usually in samples from the initials stages of purifications. To overcome Hook Effect samples to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid assay of samples containing sodium azide (NaN_3), as it may destroy the HRP activity of the conjugate resulting in the under-estimation of the levels of *P.pastoris* HCP.
4. All Standards and Samples should be assayed at least in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. 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.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards and Samples.

Preparation before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

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 **(1X) Wash Buffer**; dilute **25 ml of (20X) Wash Buffer** in **475 ml of DI water**.
4. To make **Sample Diluent (1X)**, add **10ml** of **Sample Diluent (5X)** to **40ml** of **DI water**. This is the working solution.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. Pipette **100 ul** of **Standards** or **Samples** into the respective wells.

3. Cover the plate and incubate at 37°C for 2 hours 30 minutes.

4. Aspirate and wash plate 4 times with **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. Add **100 ul** of **Anti-*P.pastoris*:HRP Conjugate** into each well.

6. Cover the plate and incubate at 37°C for 1 hour.

7. Aspirate and wash plate 4 times with **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.

8. Add **100 ul** of **TMB Substrate** in each well.

9. Incubate the plate at 37°C for 15 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

11.Read the absorbance at 450 nm with a microplate reader within 30 minutes.

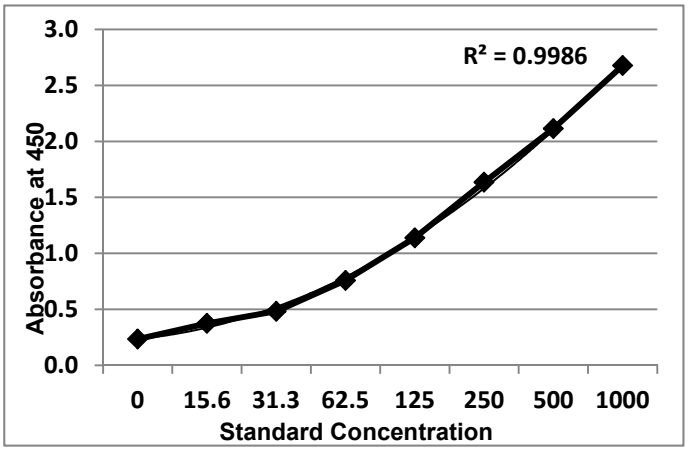
Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Subtract the mean absorbance of the zero standards (background) from each well. Using semi-log graph paper or computer programs, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown *P.pastoris* concentrations, find the unknowns 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 *P.pastoris* concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.242	0.228	0.235	1.4	--
15.6	0.371	0.377	0.374	15.0	96.5
31.3	0.490	0.475	0.482	27.1	86.6
62.5	0.756	0.761	0.759	63.5	101.6
125	1.154	1.123	1.139	129.2	103.3
250	1.626	1.645	1.635	257.7	103.1
500	2.107	2.122	2.114	470.9	94.2
1000	2.743	2.612	2.678	1031.4	103.1

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 as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays.

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 15 ng/ml.
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated and the LOD was found to be 12 ng/ml

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 (15.6 ng/ml), medium (125 ng/ml) and high (1000 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory 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%	<15%
Medium	<8%	<12%
High	<8%	<12%

Recovery/ Interference Studies:
Known amount of P.pastoris was spiked into sample diluent (1X). The resulting concentration or "recovery" of the spiked material, demonstrates if the expected value can be measured accurately. If the recovered value differs significantly from the amount expected this may be sign that some factor in the sample matrix may be causing a falsely elevated or falsely depressed value.

It was observed that sample diluent obtained the best recoveries (+/- 20%):

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 human 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.

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

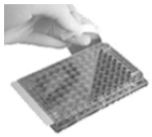
Components (2-8ºC) → 30 mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE NOW

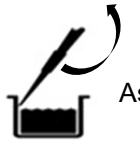
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components → AVOID → COOL (2-8ºC) → THAW (22±4ºC) → THAW (22±4ºC) → COOL (2-8ºC) → AVOID → NO CHANGE IN RESULTS

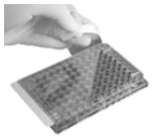
3.  Pipette **100 ul Standards/Samples** into the respective wells.

4. Cover plate  and **incubate** for 

at 37°C.

5.  Aspirate and wash wells 4 times with **(1X) Wash Buffer**.

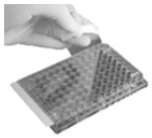
6.  Pipette **100 ul Anti P. Pastoris:HRP Conjugate** into each well.

7. Cover plate  and **incubate** for 

at 37°C.

8.  Aspirate and wash wells 4 times with **(1X) Wash Buffer**.

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and **incubate** for 

at 37°C.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	Results
1A 2A	Zero Std Zero Std			
1B 2B	15.6 ng/ml 15.6 ng/ml			
1C 2C	31.3 ng/ml 31.3 ng/ml			
1D 2D	62.5 ng/ml 62.5 ng/ml			
1E 2E	125 ng/ml 125 ng/ml			
1F 2F	250 ng/ml 250 ng/ml			
1G 2G	500 ng/ml 500 ng/ml			
1H 2H	1000 ng/ml 1000 ng/ml			
1A 2A	Sample			
1B 2B	Sample			
1C 2C	Sample			
1D 2D	Sample			

LIMITED WARRANTY

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This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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SYMBOLS KEY

MTP	Anti- *P.pastoris* Microtiter Plate (12x8 wells)
STD	*P.pastoris* Standard
HRP CONJ	*P.pastoris*:HRP Conjugate
5X SAMP DIL	(5X) Sample Diluent
20X WASH BUF	(20X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalogue Number
	Expiration Date
	Storage Temperature