KRIBIOLISA™ *E.coli* Host Cell Proteins ELISA

REF: KBBP01

Ver6.1

RUO

Immunoassay for the measurement of *E.coli* 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**

KRISHGEN BioSystems Unit Nos#318/319, Shah & Nahar, Off Dr. E Moses Road, Worli, Mumbai, Maharashtra 40001, Tel: 022 4919 8700, Email: info@krishgen.com

Introduction:

A variety of proteins and pDNA which are used as therapeutic agents in humans and animals are produced through recombinant expression in *E.coli*. The manufacturing and purification process of these products tends to leave the potential for contamination by Host Cell Proteins (HCPs) from *E.coli* 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 generic kit is intended in determining the presence of *E.coli* Host Cell Proteins contamination in various products that are manufactured through recombinant expression in *E.coli*. The kit has been validated successfully for testing of final and in process product HCPs in variety of products regardless of growth and purification process.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing *E-coli* HCPs are reacted with anti-*E.coli*:HRP antibody simultaneously in the microtiter wells already coated with affinity purified capture anti-*E.coli* antibody. This immunological reaction results in 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 (see Assay procedure section mentioned below). The substrate 3,3',5,5' Tetramethyl 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 *E.coli* HCPs present.

Materials Provided:

1. Anti-*E.coli* HCP Coated Microtiter Plate (12 x 8 wells) – 1 no
2. *E.coli* HCP Standards, (0.5 ml/vial) - 0, 5, 10, 20, 40, 60, 80 & 100 ng/ml
3. Anti-*E.coli* : HRP Conjugate - 12 ml
4. Wash Buffer (20X) - 25 ml
5. Sample Diluent - 20 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 12 ml
8. 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 pipettes to measure volumes ranging from 25µl to 1000µl.
3. Distilled water.
4. Wash bottle or automated microplate washer.
5. Semi log graph paper or software for data analysis.
6. Timer.
7. Absorbent paper.

Handling/Storage:

1. All reagents should be stored at 2^0C to 8^0C for stability.
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 ºC). Upon assay completion return all components to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. All the reagents provided may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing use only.

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

1. Bring all reagents to Room Temperature before use.
2. To make Wash Buffer (1X), dilute 20ml of Wash Buffer (20X) in 380ml of DI water and store at 4^0C.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
2. If the 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 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 *E-coli* 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.

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. Add 50µl of **Sample Diluent** and 100 µl **Standards** and **Samples** into the respective wells as mentioned in the worklist.

3. Cover the plate and incubate it for 2 hour at 37°C.

4. Aspirate and wash plate 5 times and 30 sec soak time 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. All the washes should be performed similarly.

5. Add 50µl of **Sample Diluent** and 100µl of **Anti-*E.coli* : HRP Conjugate** into each well.

6. Cover the plate and incubate it for 2 hour at 37°C.

7. Aspirate and wash plate 5 times and 30 sec soak time with **Wash Buffer (1X)** as mentioned in the Step No 4.

6. Pipette out 100µl of **TMB Substrate** in each well.

7. Incubate the plate at 37°C for 30 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color

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

9. Read the absorbance at 450/630nm blanking on the Zero Standard.

Example of a Work list

Well #	Contents	Abs at 450	Mean Absorbance	ng/ml HCP equivs
1A 2A	Zero Std Zero Std			
1B 2B	5 ng/ml 5 ng/ml			
1C 2C	10 ng/ml 10 ng/ml			
1D 2D	20 ng/ml 20 ng/ml			
1E 2E	40 ng/ml 40 ng/ml			
1F 2F	60 ng/ml 60 ng/ml			
1G 2G	80 ng/ml 80 ng/ml			
1H 2H	100 ng/ml 100 ng/ml			

Calculation of Results:

It is recommended to use the data reduction program (logit-log) in the reader to determine the HCP concentration in the unknown samples. Plot the % bound on the vertical axis (logit) against the HCP concentrations on the horizontal axis (log) for each standard (except the zero standard).

Alternatively, calculate percent conjugate bound (%B) for each standard, and sample relative to the maximum binding (B_0, Zero standard) wells as follows:

$$\%B/B_0 = \frac{\text{Mean Absorbance (highest standard) - Mean Absorbance (standard/sample)}}{\text{Mean Absorbance (highest standard) - Mean Absorbance (Zero standard)}} \text{ X } 100$$

Using semi-log graph, plot % bound on the vertical axis against HCP concentration on the horizontal axis for each of the calibrators and draw a smooth line curve through the points. HCP concentrations for the unknown may then be estimated from the line by interpolation.

Of the methods surveyed the logistic-log and fully specified logit-log functions are the most accurate models for forming standard curves and for interpolating HCP concentrations from the standard curve. The accuracy of the fully specified logit-log function is highly dependent on the precise specification of two unknown quantities, the optical densities at zero and infinite concentrations, prior to fitting the model to a typical set of calibration data. The function does not require pre-specification of any parameters before estimating the standard curve, and the four parameters are readily interpretable in terms of identifiable physical quantities. This model also has the advantage that it is easiest to visualize since it does not incorporate complex transformations of the optical density scale.

Performance Characteristics:

1. **Sensitivity:**

a) **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 5 ng/ml

b) **Limit of Quantitation:** It is defined as the lowest concentration for which Coefficient of Variation is <20%. The LOQ is found to be <6 ng/ml.

2. **Specificity / Cross reactivity:** Specificity of an analytical method is defined as its ability to measure an analyte accurately in the presence of interference**.**
   Antibodies used in the kit have been tested for reactivity with more than 50 proteins by Western Blot. Western Blot analysis against several strains of E.coli (XLI- Blue, DH5α, BL21, JM109, JM110, top 10, K12 & MC 1061) indicate that most of the proteins are conserved among all strains. Cross reactivity has not been specifically investigated with this Kit.

3. **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 (8 ng/ml) and high (80 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	5.96%	13.47%
High	3.84%	7.48%

4. **Recovery by Spiking:** In spike and recovery, a known amount of analyte is added (spiked) into the natural test sample matrix and its response is measured(recovered) in the assay by comparision to an identical spike in the standard diluent.

Sample Buffer Matrix	Pure Antigen Added (ng/ml)	Expected	Observed	Recovery (%)
Sample Diluent	10	10	10.5	105%
	20	20	21.5	107.5%
	40	40	29	97.5%
	80	80	79	98.8%
Stabilzyme Noble Stabilizer (BSA free)™	10	10	9	90%
	20	20	16	80%
	40	40	32	80%
	80	80	70	88%
Assay Diluent	10	10	12	120%
	20	20	20	100%
	40	40	40	100%
	80	80	82	103%

Stabilzyme Noble™ is registered trade mark of Surmodics, USA.

5. **Standard Curve Characteristics:**

Assays	Correlation Coefficient (r)	Intercept (A)	Slope (B)
Assay	**0.994**	**6.33**	**-3.61**

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.

Safety Precautions:

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.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

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

Krishgen Biosystems. 2019

THANK YOU FOR USING KRISHGEN PRODUCT!