



KRIBIOLISA *E.coli* Host Cell Proteins ELISA

Catalog No: KBBP01

Immunoassay for the measurement of *E.coli* Host Cell Proteins

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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 *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 Protein contamination in various products that are manufactured through recombinant expression in *E.coli*. The kit has been validated successfully for testing of 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 *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* coated Microtitre plate (96 wells) – 1 no
2. Anti-*E.coli*:HRP, 11mL – 1 bottle
3. *E.coli* HCP Standards, (0.5mL/vial) – 0, 1, 3, 12, 40 & 100 ng/mL
4. TMB Substrate, 12mL – 1 bottle
5. Wash Buffer (10X), 100 mL – 1 bottle
6. Stop Solution, 12mL – 1 bottle
7. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm and 630 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 μ L to 100 μ L.
3. Distilled water.
4. Wash bottle or automated microplate washer.
5. Log-Log graph paper or software for data analysis.
6. Timer.
7. Absorbent paper.
8. 1 Liter bottle for diluted Wash Buffer

Handling/Storage:

1. All reagents should be stored at 2^oC to 8^oC for stability.
2. All the reagents and wash solution are stable until the expiration date of the kit.
3. The substrate reagent should not be used if its stopped absorbance at 450nm is greater than 0.2
4. Before using, bring all components to room temperature (18-25 °C). Upon assay completion return all components to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives 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. Label any aliquots made with the kit Lot No and expiration date and store it at appropriate conditions mentioned.
2. Dilute Wash Buffer to 1 Liter in Distilled water and store at 4^oC.
3. Bring all reagents to room temperature before use.

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. High Dose Hook Effect may be observed in samples with very high concentrations of HCP. High Dose Hook Effect is due to insufficient excess of antibody for very high concentrations of HCPs present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the HCP concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of samples containing sodium azide (NaN₃), 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, Controls and Samples should be assayed atleast 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, Controls 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. Pipette out 25 µL of **Standards**, **Controls** and **Samples** into the respective wells as mentioned in the worklist.
3. Pipette out 100 µL of anti-*E.coli*:HRP **Conjugate** into each well.
4. Cover the plate and incubate it on a plate shaker at ~180rpm for 90 minutes at room temperature, 22°C±4° or incubate for about 150 minutes without shaking.
5. Aspirate and wash plate 4 times with **Wash Buffer** 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.
6. Pipette out 100 µL of TMB **Substrate** in each well.
7. Incubate the plate at room temperature 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 100 µL 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 450/630nm	Mean Absorbance	ng/ml HCP equivs
1A	Zero Std			
1B	Zero Std			
1C	1 ng/mL			
1D	1 ng/mL			
1E	3 ng/mL			
1F	3 ng/mL			
1G	12 ng/mL			
1H	12 ng/mL			
2A	40 ng/mL			
2B	40 ng/mL			
2C	100 ng/mL			
2D	100 ng/mL			
2E	Sample A			
2F	Sample A			
2G	Sample B			
2H	Sample B			

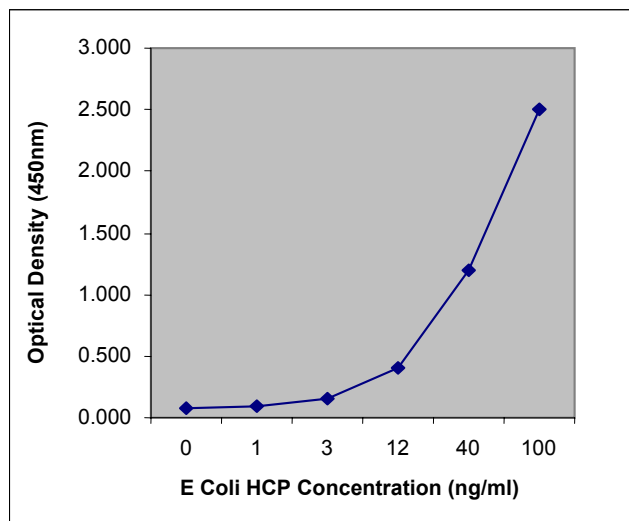
Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards, Controls and Samples. Subtract the Mean Absorbance of the Zero Standards (background) from each well. Plot the standard curve on lin-lin graph paper, with Standard HCP Concentration on the X-axis and Absorbance on the Y-axis. Draw the best fit straight line through the standard points.

To determine the unknown 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 HCP Concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software is preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.



Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

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:

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' standard were evaluated and the LOD was found to be 5 ng/well equivalent to 200ng/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 <5 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 and technician to technician, it is recommended that all operators achieve precision as per 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 comparison to an identical spike in the standard diluent.

Sample Buffer Matrix	Pure Antigen Added (ng/ml)	Expected	Observed	Recovery (%)
Sample Diluent	10.0	14.8	15.0	101 %
	20.0	24.8	23.0	93 %
	40.0	44.8	44.0	98 %
	80.0	80.8	79.0	93 %
Assay Diluent	10.0	10.0	12.0	120 %
	20.0	20.0	20.0	100 %
	40.0	40.0	40.0	100 %
	80.0	80.0	82.0	103 %

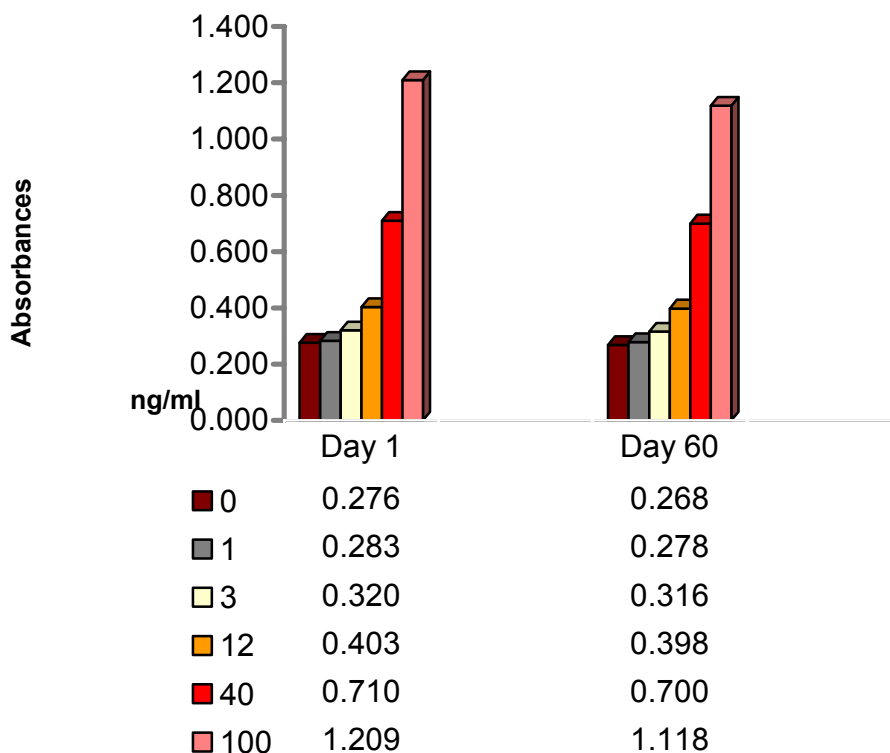
5. Wet and Dry Assay Validation:

Assays	Correlation Coefficient (r)	Intercept (A)	Slope (B)
Wet Assay	0.994	4.79	-2.388
Dry Assay	0.994	6.33	-3.610

Wet Assay was typically run after the kit was manufactured prior to stabilization of the coated microplate with proprietary reagents for long term storage.

Dry Assay was run after the kit was manufactured and the coated microplate stabilized with proprietary reagents for long term storage.

6 Accelerated Shelf Life Study :



Absorbance at Day 60 has been done using storage of coated microtitre plate at elevated temperature of 45°C. This time temperature relationship has been established to affix the criteria of the expiration date of this kit based on the data generated by us and at recommended storage conditions.

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.

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