



***E. coli* Host Cell Protein ELISA Kit**

1 Plate Kit Catalog # AB000105

Complete kit for the determination of *E. coli* host cell protein contamination in bulk products expressed in *E. coli* expression systems.

Please read this insert completely prior to performing the assay.

This kit is intended for research use only. Not for use in diagnostic procedures.

Assay Principle

The *E. coli* Host Cell Protein (HCP) ELISA kit is designed to quantitatively measure host cell protein contamination in bulk products expressed in *E. coli* expression systems. Please read the complete kit insert before performing this assay. A series of *E. coli* protein standards are made from a provided stock solution and used to generate a standard curve for the assay and all unknown sample concentrations should be read off this standard curve. *E. coli* standards or diluted unknown samples are pipetted into the provided 96-well plate which has been pre-coated with anti-*E. coli* HCP antibodies to capture *E. coli* proteins from biologics samples. Following an incubation to allow capture of the *E. coli* protein by the antibodies on the plate, a second anti-*E. coli* HCP antibody, conjugated with biotin, is added and incubated to allow it to bind to the captured *E. coli* proteins. After a 45 minute incubation, the plate is washed and a Streptavidin-HRP (Horse Radish Peroxidase) conjugate is added and incubated for 30 minutes. The Streptavidin-HRP conjugate will be captured by any biotin labeled antibody bound to the plate. Following a wash step to remove unbound conjugate, TMB substrate is added and is converted by the captured HRP to a colored product in proportion to the amount of HCP bound to the plate. After a ten minute incubation to allow color development, the reaction is stopped and the intensity of the generated color is detected in a spectrophotometer plate reader capable of measuring 450nm wavelength. A standard curve will be generated from the *E. coli* protein standards and used to calculate the concentration of *E. coli* proteins in the unknown samples, after making suitable correction for the dilution of the sample.

Supplied Components

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) coated with Rabbit anti-*E. coli* HCP IgG.
Kit AB-000105-1 (1 plate)

E. coli Protein Standards

600µl of *E. coli* proteins at a concentration of 2430 ng/ml, sufficient for generating a standard curve from 3.3 ng/ml to 810 ng/ml.
Kit AB-000105-1 (1 tube with HCP stds and 7 dilution tubes)

5x Dilution Buffer

Buffer used for dilution of antibodies and Streptavidin-HRP conjugate. The 15 ml of concentrate should be diluted to 75 ml with 60 ml of deionized or distilled water.
Kit AB-000105 (15 ml)

10x PBS-T

After dilution, this is used for wash solution (PBS with 0.1% Tween-20). The 30 ml of concentrate should be diluted to 300 ml with 270 ml of deionized or distilled water.
Kit AB-000105 (30 ml)

Reporting antibody

A biotin labeled Rabbit polyclonal antibody specific for *E. coli* cell proteins. During the assay, dilute 75 µl into 15 ml of 1x Dilution buffer to give a 5 µg/ml working stock.
Kit AB-000105 (1 mg / ml, 150 µL / tube)

Streptavidin-HRP Conjugate

A Streptavidin – Horse Radish Peroxidase conjugate in a special stabilizing solution. During the assay, spin the tube briefly and then dilute the entire 375 µl into 15 ml of 1x Dilution buffer to give a 0.1 µg/ml working stock.
Kit AB-000105 (4 µg / ml, 375 µL / tube)

TMB Substrate

Use directly without dilution.
Kit AB-000105 (15 ml)

Stop Solution

A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.
Kit AB-000105 (15 ml)

Plate Sealer

Kit AB-000105-1 (one)

Other Materials Required

Distilled or deionized water.

Single- and multi-channel micro-pipettes with disposable tips to accurately dispense volumes 5-250 μ L.

Plastic tubes (i.e. 1.5 ml) for sample dilution

Reagent reservoirs for sample addition

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4-parameter) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 2

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Procedural Notes

Allow diluted reagents and buffers to reach room temperature (18-25°C) prior to starting the assay. Once the assay has been started, all steps should be completed in sequence and without interruption. You do not want the plate to dry out in between steps as this can cause high backgrounds or erroneous results. Make sure that required reagents and buffers are ready when needed. Prior to adding to the plate, reagents should be mixed gently (not vortexed) by swirling.

Avoid contamination of reagents, pipette tips and wells. Use new disposable tips and reservoirs, do not return unused reagent to the stock bottles/vials and do not mix caps of stock solutions.

Incubation time can affect results. All wells should be handled in the same order for each step.

Microplate washing is important and can affect results by giving erroneous results or high backgrounds. We recommend a multichannel pipette to add 250 µl of buffer to each well across the plate, followed by a dumping out of contents (to a sink or other receptacle) with a rapid wrist motion. The plate should then be tapped firmly on a paper towel to shake out any remaining liquid. Avoid prolonged incubation in wash buffer when performing wash steps.

When making additions to the plate, be careful to avoid damaging the coating, for example by scratching the bottoms or the sides of the wells. One technique to avoid this is to make additions (for a right-handed person) from left to right across the plate, supporting the pipette tips on the right edge of the well with each addition and thus avoiding contact with the bottom or sides of the wells.

During the incubation times, the plate should be covered to minimize evaporation from the wells. This can be done with the adhesive covers provided or by stacking an empty plate on top.

After the last wash step and prior to adding the TMB substrate, wipe the bottom of the plate with a clean paper towel to ensure that moisture or fingerprints do not interfere with the OD reading.

Once the TMB substrate is added it will be converted by the captured HRP to a blue colored product. Generally we find that a 10 to 15 minutes incubation is sufficient for enough color development to discern differences between the standards and the reaction should be stopped at this point. Bear in mind that, given sufficient time, even a small amount HRP is capable of converting all the TMB to product and if this happens it will be difficult to discern differences between differing concentrations of HCP. Keeping OD₄₅₀ values well below 2.0 will result in greatest accuracy as at high absorbance values very little light is reaching the detector and measurements are error prone. (Remember that at an OD of 1.0 only 10% of the light is being detected and at an OD of 2.0 only 1% of the light is reaching the detector).

Assay Protocol

1. Use the plate layout sheet on the back page to plan sample layout on plate and also aid in proper sample and standard identification after the assay. The plate layout sheet has been pre-populated with suggested positions for the standards. We recommend that assays are carried out in duplicate or (preferably) triplicate in order to minimize spurious results.
2. Dilute the 10x PBS-T and 5x Dilution buffer with water to 1x-strength. Check both concentrate bottles for precipitates before proceeding and if found warm slightly in a water bath to dissolve before proceeding. The 30 ml of 10xPBS-T should be diluted to 300 ml with 270 ml milliQ water. The 5x dilution buffer can be diluted in a disposable 50ml tube, adding 10ml of the 5x solution to 40ml of milliQ water. If you need more than 50ml you can dilute the remaining 5ml of 5x stock.
3. Prepare the HCP standards by numbering seven 1.5ml tubes and adding 1ml of Dilution buffer to each. Cap the seventh tube....this will be the blank (0ng/ml HCP). To tube one add 500ul of the provided 2430ng/ml HCP stock and mix well...this will be the 810 ng/ml standard. Then serially dilute 500µl of tube one across tubes two through six to generate the remainder of the standards. Pipette 100ul of each standard into the plate according to the plate map.
4. Dilute your sample in 1x dilution buffer; for best accuracy perform serial dilutions over a wide range such that multiple dilutions will span the range of 10 – 800 ng/ml. For drug substance the typical testing protein concentration is 2 mg/ml; it is not recommended to test drug substance at concentrations higher than 4 mg/ml. Pipette 100 µL of samples or standards into wells in the plate. Cover plate and incubate 1.5 hour at room temperature.
5. During the above incubation, dilute the 1 mg/ml reporting antibody to 5 µg/ml by adding 75 µL to 15 ml of 1x Dilution Buffer.
6. Wash plate by emptying contents and adding 250 µL of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
7. Pipette 100 µL of Reporting Antibody into each well. Cover plate and incubate plate 45 minutes at room temperature.
8. During the above incubation, dilute the 4 µg/ml Streptavidin-HRP conjugate to 0.1 µg/ml by adding the entire 375 µL to 15 ml of 1x Dilution Buffer.

9. Wash plate by emptying contents and adding 250 μ L of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
10. Pipette 100 μ L of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 30 minutes at room temperature.
11. Wash plate by emptying contents and adding 250 μ L of wash buffer to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. Repeat twice.
12. Add 100 μ L of TMB substrate to each well. Monitor color development and stop reaction by adding 100 μ L of Stop Solution to each well when color development within standards is sufficient. Generally 10 minutes time will be sufficient; incubating longer will increase the background.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm, use three wells without sample as blank such as H1-H3.
14. Either graph the results on log graph paper or use the plate reader's built-in 4-parameter fit software capabilities to calculate HCP concentration for each unknown sample.

Calculation of Results

Average the triplicate OD readings for each standard, sample and background wells to give a mean OD reading. Subtract the averaged background values from the mean OD values to give a net OD value and create a standard curve using either log graph paper or 4-parameter fit software. Match OD values for the unknowns to [HCP] using the standard curve, remembering to correct for dilution.

Sample data

Sample	Mean OD	Actual [HCP]	Calculated [HCP]
810 ng/ml HCP	2.583	810	814
270ng/ml HCP	1.946	270	270
90 ng/ml HCP	1.168	90	91
30 ng/ml HCP	0.673	30	30
10 ng/ml HCP	0.483	10	9.0
3.3 ng/ml HCP	0.440	3.3	4.0
NSB (0ng/ml HCP)	0.414	0	3.6
Unknown 1			
Unknown 2			
Unknown 3			

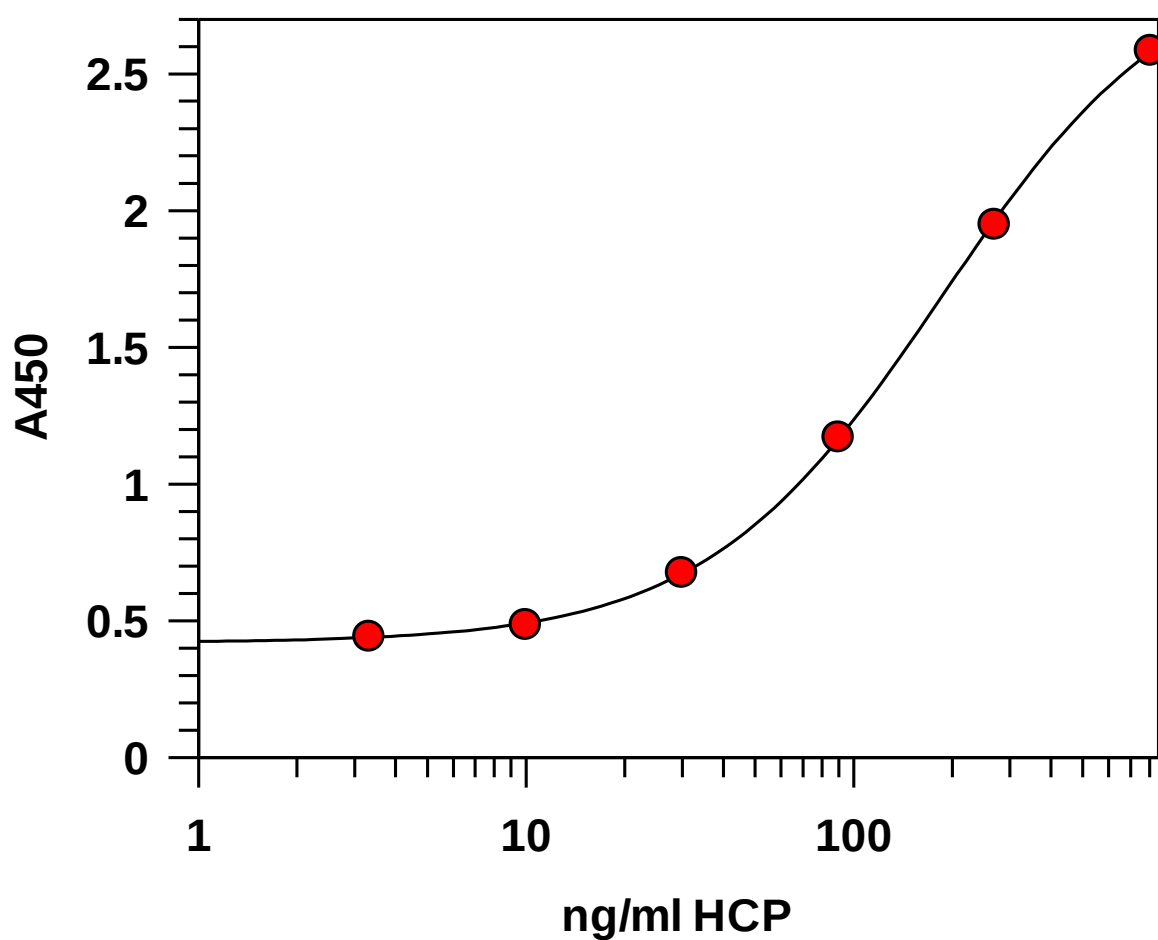


Plate Template

	1										
A	810ng/ml std	810ng/ml std	810ng/ml std								
B	270ng/ml std	270ng/ml std	270ng/ml std								
C	90ng/ml std	90ng/ml std	90ng/ml std								
D	30ng/ml std	30ng/ml std	30ng/ml std								
E	10ng/ml std	10ng/ml std	10ng/ml std								
F	3.3ng/ml std	3.3ng/ml std	3.3ng/ml std								
G	0ng/ml std	0ng/ml std	0ng/ml std								
H											