Product Manual

E. coli Host Cell Protein ELISA Kit

Catalog #: ENZ-KIT127

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

NOTE: This version contains a change to the kit storage conditions.

For the latest product information, including support documentation, visit us online:
www.enzolifesciences.com

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

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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 is provided 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.

MATERIALS SUPPLIED

Coated Clear 96 Well Plates

A clear plastic microtiter plate(s) with break apart strips coated with Rabbit anti-*E. coli* HCP IgG.

(1 plate)

E. coli Protein Standards

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.

(600 µL, 1 tube with HCP standard)

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.

(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.

(30 mL)

Reporting antibody

A biotin labeled Rabbit polyclonal antibody specific for *E. coli* cell proteins. During the assay, dilute 120 µL into 12 mL of 1x Dilution buffer.

(135 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.

(4 µg/mL, 400 µL/tube)

TMB Substrate

Use directly without dilution.

(15 mL)

Stop Solution

A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution.

(15 mL)

Plate Sealer

(one)

OTHER MATERIALS REQUIRED

1. Distilled or deionized water.
2. Single- and multi-channel micro-pipettes with disposable tips to accurately dispense volumes 5-250 µL.
3. Plastic tubes (i.e. 1.5 mL) for sample and standard dilution
4. Reagent reservoirs for sample addition
5. Colorimetric 96 well microplate reader capable of reading optical density at 450nm.
6. 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.

STORAGE AND STABILITY

All components of this kit are shipped at 4°C. Upon receipt, the whole kit can be stored at 4°C. Alternatively, the kit components can be stored at the temperature indicated on each component label. When stored as indicated, all components are stable until the kit's expiration date. Shipping conditions may not reflect final storage conditions.

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 3.

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 is 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 OD_{450} 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).

SAMPLE PREPARATION SUGGESTIONS

For drug substance, it is recommended to use 4 mg/mL or less for the HCP quantification; method qualification and ICH validation should also use the same recommended protein concentration. For in-process samples running for the first time, pre-rProtein A column fraction needs to be serially diluted 4 times using a 1:10 dilution, one or two of the dilutions will have HCP levels that fall onto the HCP standard curve. After the first test, it is sufficient to test at one dilution where the HCP readings fell in the range of 10-270 ng/mL in the standard curve. For the post-rProtein A fraction, we recommend you serially dilute twice with 1:10 dilution. For any downstream samples (cation exchange and anion exchange column fraction), the original sample and a 1:10 dilution sample should be tested for the first test. For spike recovery testing in drug substance, it is recommended to use 20-30 ng/mL HCP whereas for in-process samples a 100 ng/mL spike is recommended. We also point out that the HCP ELISA may not achieve linear dilution for drug substances because of the complexity of the drug composition whereas for in-process samples, linear dilution can be expected.

ASSAY PROTOCOL

1. Use the plate layout sheet on page 10 to plan sample layout on plate and also aid in proper sample and standard identification after the assay. 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 water and the 5x Dilution buffer can be diluted in a disposable 50 mL tube, adding 10 mL of the 5x solution to 40 mL of water. If you need more than 50 mL, you can dilute the remaining 5 mL of 5x stock.
3. Prepare the HCP standards by numbering seven 1.5 mL tubes and adding 1 mL of Dilution buffer to each. Cap the seventh tube. This will be the blank (0 ng/mL HCP). Add 500 µL of the provided 2430 ng/mL HCP stock to tube one and mix well. This will be the 810 ng/mL standard. Then serially dilute 500 µL of tube one across tubes two through six to generate the remainder of the standards. Pipette 100 µL 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. 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 reporting antibody to by adding 120 µL to 12 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 min. 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 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.

10. Pipette 100 µL of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 30 min. at room temperature.
11. 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.
12. Add 100 µL of TMB substrate to each well. Monitor color development and stop reaction by adding 100 µL 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 450nm, 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 of 4-parameter fit software. Match OD values for the unknowns to [HCP] using the standard curve, remembering to correct for dilution.

Data

Sample	Mean OD	[HCP]
NSB (0ng/mL HCP)		
1.1 ng/mL HCP		
3.3 ng/mL HCP		
10 ng/mL HCP		
30 ng/mL HCP		
90 ng/mL HCP		
270ng/mL HCP		
810ng/mL HCP		
Unknown 1		
Unknown 2		
Unknown 3		

Plate 1 Template

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								

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