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ab240997 *E. coli* HCP ELISA Kit (host cell protein)

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

This product is for research use only and is not intended for diagnostic use.

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1. Overview

E. coli HCP ELISA Kit (host cell protein) (ab240997) is designed to quantitatively measure host cell protein (HCP) contamination in bulk products expressed in *E. coli* expression systems.

The provided 96-well plate has been pre-coated with Rabbit anti-*E. coli* HCP antibodies to capture *E. coli* proteins from biological 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 incubation, the plate is washed and a Streptavidin-HRP conjugate is added and incubated for 30 min. 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 short 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.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed.



Add 100 μ L standard or sample to appropriate wells. Cover and incubate for 90 min at room temperature.



Discard incubation solution and wash plate 2 times with 250 μ L Wash Buffer.



Add 100 μ L Reporting Antibody to each well. Cover and incubate for 45 min at room temperature.



Discard the solution and wash plate 2 times with 250 μ L Wash Buffer.



Add 100 μ L Streptavidin-HRP conjugate. Cover and incubate for 30 min at room temperature.



Discard incubation solution and wash plate 2 times with 250 μ L Wash Buffer.



Add 100 μ L TMB Substrate. Monitor color development. 10 min should be sufficient.



Add 100 μ L Stop Solution and read OD at 450 nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors.

6. Materials Supplied

Item	Quantity	Storage
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		condition
Coated Clear 96 Well Plate	1 unit	+4°C
<i>E. coli</i> Protein Standard	600 µL	+4°C
5X Dilution Buffer	15 mL	+4°C
10X PBST	30 mL	+4°C
Plate Sealer	1 unit	+4°C
Reporting Antibody	150 µL	+4°C
Stop Solution (1M H ₂ SO ₄)	15 mL	+4°C
Streptavidin-HRP Conjugate	400 µL	+4°C
TMB Substrate	15 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 450 nm
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for sample dilution.

8. Technical Hints

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- Avoid multiple freeze/thaw of samples.

- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- 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.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, reagents should be mixed gently (not vortexed) by swirling.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 5X Dilution Buffer:

Check concentrate bottle for precipitates before proceeding and if found warm slightly in a water bath to dissolve. Dilute to 75 mL with 60 mL of deionized water to make 1X Dilution Buffer.

9.2 10X PBST:

Check concentrate bottle for precipitates before proceeding and if found warm slightly in a water bath to dissolve. Dilute to 300 mL with 270 mL of deionized water. After dilution, this is used for wash solution (PBS with 0.1% Tween-20).

9.3 Reporting Antibody:

Immediately prior to the assay, dilute the 1 mg/mL reporting antibody to 5 µg/mL by adding 75 µL to 15 mL of 1X Dilution Buffer.

9.4 Streptavidin-HRP Conjugate:

Immediately prior to the assay, dilute 375 µL into 15 mL of 1X Dilution buffer to give a 0.1 µg/mL working stock. (4 µg/mL, 420 µL/tube).

10. Standard and Control Preparation

Prepare the HCP standards by numbering seven 1.5 mL tubes and adding 1 mL of Dilution buffer to each. Cap the 7th tube, this will be the blank (0 ng/mL HCP).

Standard #	Dilution Buffer	<i>E. coli</i> Protein Standard stock (2430 ng/mL)	<i>E. coli</i> Protein Standards (ng/mL)
1	1 mL	500 μ L	810
2	1 mL	500 μ L of tube 1	270
3	1 mL	500 μ L of tube 2	90
4	1 mL	500 μ L of tube 3	30
5	1 mL	500 μ L of tube 4	10
6	1 mL	500 μ L of tube 5	3.3
7	1 mL	0	0

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

11.1 Drug substance:

For drug substance, it is recommended to use 4mg/mL or less for the HCP quantification; method qualification and ICH validation should also use the same recommended protein concentration.

11.2 In-process samples:

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.

11.3 Downstream samples:

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.

11.4 Spike recovery testing:

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.

12. Assay Procedure

- Prepare reagents within 30 minutes before the experiment.
 - Equilibrate all materials and prepared reagents to room temperature 15 minutes prior to use.
 - 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.
 - We recommend that you assay all standards, controls and samples in duplicate.
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- 12.1 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.
 - 12.2 Pipette 100 μ L of samples or standards into wells in the plate. Cover plate and incubate 90 min at room temperature.
 - 12.3 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.
 - 12.4 Pipette 100 μ L of Reporting Antibody into each well. Cover plate and incubate plate 45 min at room temperature.
 - 12.5 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.
 - 12.6 Pipette 100 μ L of Streptavidin-HRP conjugate into wells. Cover plate and incubate plate 30 min at room temperature.
 - 12.7 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.
 - 12.8 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 min time will be sufficient. Longer incubation times may increase background.

- 12.9 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.
- 12.10 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 sample.

Δ Note: 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 min 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 of 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).

13. Calculations

- 13.1 Average the OD readings for each standard, sample and background wells to give a mean OD reading.
- 13.2 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.
- 13.3 Match OD values for the unknowns to [HCP] using the standard curve, remembering to correct for dilution.

14. Notes

Technical Support

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