PRODUCT MANUAL HCP-002

E. COLI HOST CELL PROTEIN ELISA KIT

E. COLI HCP ELISA KIT

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

INCLUDED

- Coated 96-Well Strip Plate
- E. coli Protein Standard
- 5x Dilution Buffer
- 10x PBS-T
- Reporting Antibody
- Streptavidin-HRP Conjugate
- TMB Substrate
- Stop Solution
- Plate Sealer

ASSAY PRINCIPLE

The E. coli Host Cell Protein (HCP) ELISA kit is designed to quantitatively measure HCP contamination in bulk products expressed in E. coli expression systems. A vial of concentrated E. coli protein is provided to generate a standard curve for the assay. E. coli standards or unknown samples are pipetted into the provided 96-well strip 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. Next, a Streptavidin-HRP conjugate is added and will be captured by any biotin labeled antibody bound to the plate.TMB substrate is added and converted by the captured HRP to a colored product in proportion to the amount of HCP bound to the plate. The reaction is stopped and the intensity of the generated color is detected in a spectrophotometer plate reader capable of measuring 450 nm wavelength. A standard curve should be generated from the E. coli protein standards and used to calculate the concentration of E. coli proteins in the unknown samples, taking into account any unknown sample dilution made. A pilot experiment may be run first to determine the optimal dilution of your sample so that the sample falls within the linear range of the standard curve.

Note: sodium azide will interfere with this assay and should not be used in samples or buffers.

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SUPPLIED COMPONENTS

ENTIRE KIT MUST BE STORED AT 4°C.

Clear 96-Well Strip Plate
Clear plastic strip-well microtiter plate coated with rabbit anti-E. coli HCP IgG. Can be used as individual strips.

E. coli Protein Standard (2430 ng/ml, 600 µl)
Concentrated E. coli proteins sufficient for generating a standard curve from 810 ng/ml to 1.1 ng/ml.

5x Dilution Buffer (15 ml)
1x Dilution Buffer is used for dilution of Reporting Antibody and Streptavidin-HRP conjugate. 1x Dilution Buffer is used to dilute samples if necessary. The 10 ml of 5x concentrate should be diluted to 50 ml with 40 ml of milliQ water to achieve 1x Dilution Buffer.

10x PBS-T (30 ml)
1x PBS-T is used for wash steps. The 25 ml of 10x concentrate should be diluted to 250 ml with 225 ml of milliQ water to achieve 1x PBS-T.

Reporting Antibody (135 µl/tube)
A biotin labeled rabbit polyclonal antibody specific for E. coli cell proteins. Immediately prior to the assay, dilute 120 µl into 12 ml of 1x Dilution Buffer.

Streptavidin-HRP Conjugate (4 µg/ml, 420 µl/tube)
A Streptavidin–Horse Radish Peroxidase conjugate in a stabilizing solution. Immediately prior to the assay, dilute 375 µl into 15 ml of 1x Dilution Buffer to give a 0.1 µg/ml working stock.

TMB Substrate (15 ml)
Use directly without dilution.

Stop Solution (15 ml)
A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

Plate Sealer

OTHER MATERIALS REQUIRED

milliQ water

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

1.5 ml eppendorf tubes

Reagent reservoirs for sample addition

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

ASSAY PROTOCOL

1. Dilute the 10x PBS-T and 5x Dilution Buffer to 1x-strength with milliQ water. The 25 ml of 10x PBS-T should be diluted to 250 ml with 225 ml milliQ water. 10 ml of 5x Dilution Buffer should be diluted to 50 ml with 40 ml of milliQ water.

2. Prepare the HCP standards by numbering eight 1.5 ml tubes and adding 1ml of Dilution Buffer to each. Cap the eighth tube, this will be the blank (0 ng/ml HCP). To tube one add 500 µl of the provided 2430 ng/ml HCP stock and mix well, this will be the 810 ng/ml standard. Then serially dilute 500 ml of tube one across tubes two through seven to generate the remainder of the standards. Pipette 100 µl of each standard and the blank into the plate.

3. Pipette 100 µl of samples into wells in the plate. If necessary, first dilute the samples in 1x Dilution Buffer.

4. Cover plate with individual plate seal and incubate **1.5 hours** at room temperature.

5. During the above incubation, dilute the Reporting Antibody by adding 120 µl to 12 ml of 1x Dilution Buffer.

6. Wash plate by emptying contents and adding 250 µl of 1x PBS-T to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. **Repeat 1x PBS-T wash step two additional times.**

7. Pipette 100 µl of Reporting Antibody into each well. Cover plate with the plate seal 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 375 µl to 15 ml of 1x Dilution Buffer.

9. Wash plate by emptying contents and adding 250 µl of 1x PBS-T to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. **Repeat 1x PBS-T wash step two additional times.**

10. Pipette 100 µl 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 µl of 1x PBS-T to each well. Empty wells again and tap the plate firmly upside down on a paper towel to fully empty well. **Repeat 1x PBS-T wash step two additional times.**

12. Add 100 µl of TMB substrate to each well. Monitor color development. Generally 10 minutes time will be sufficient; incubating longer may increase the background.

13. Stop reaction by adding 100 µl of Stop Solution to each well containing TMB when the color development within standards is sufficient.

14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm. A standard curve should be generated from the E. coli protein standards and used to calculate the concentration of E. coli proteins in the unknown samples, taking into account any unknown sample dilution made.

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