

HCP SELECT E. COLI Ab SELECTION KIT

Complete kit for the identification of the top performing antibody pair for your E. coli line.

INCLUDED

- 96-well strip plate coated with 4 different antibodies
- E. coli Protein Standard
- 5x Dilution Buffer
- 10x PBS-T
- 4 Reporting Antibodies
- Streptavidin-HRP Conjugate
- TMB Substrate
- Stop Solution
- Plate Sealer

PRODUCT MANUAL HCP-102

HCP SELECT E. COLI ANTIBODY SELECTION ELISA

ASSAY PRINCIPLE

The HCP Select E. coli Antibody Selection ELISA kit is designed to screen biologics samples to identify the best matching antibody pairs for the sensitive and quantitative measure of host cell protein contamination in bulk products expressed in E. coli expression systems. Four different antibody pairs (coating and reporting antibody) have been carefully chosen to recognize very different E. coli HCP profiles based on 1-D and 2-D total protein analysis. The HCP Select E. coli ELISA allows the identification of an assay with a more sensitive detection of HCPs from the bioprocess samples than with a single generic kit. It is recommended to test at least the drug substance and post-Protein A column fraction to provide a good read on the sensitivity of the antibody pairs.

Four different antibodies (C1, C2, C3 and C4) are coated to different areas of the microplate (please refer to the microplate diagram for details). After adding the 4 corresponding HCP standards and testing sample, the captured HCPs will be recognized by the 4 different reporting antibodies (R1, R2, R3 and R4). A series of E. coli protein standards are prepared according to the instruction to generate 4 standard curves for the assay and all unknown sample concentrations should be read off these standard curves separately.

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 45 min 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 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. 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.

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

SUPPLIED COMPONENTS

ENTIRE KIT MUST BE STORED AT 4°C.

Clear 96-Well Strip Plate

A clear plastic microtiter plate(s) coated with Rabbit anti-E. coli HCP IgG, columns 1-3 are coated with C1; columns 4-6 are coated with C2; columns 7-9 are coated with C3 and columns 10-12 are coated with C4.

E. coli Protein Standard (2430 ng/ml, 600 µl)

4 sets of 600µl 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 (90µl/tube)

4 sets of biotin labeled rabbit polyclonal antibody specific for 4 sets of E. coli cell proteins. Immediately prior to the assay, dilute 50 µl into 5 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 μ l.

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 30 ml of 10x PBS-T should be diluted to 300 ml with 270 ml milliQ water. 15 ml of 5x Dilution Buffer should be diluted to 75 ml with 60 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 μ l of the provided 2430 ng/ml HCP stock and mix well, this will be the 810 ng/ml standard. Then serially dilute 500 μ l of tube one across tubes two through seven to generate the remainder of the standards. Prepare all four sets of standards before adding to plate. Pipette 100 μ l of each standard and the blank into the plate.
3. Dilute samples in 1x dilution buffer (for screening purposes, it is recommended to test the drug substance and post-Protein A column fraction in duplicates); for best accuracy perform serial dilutions over a wide range such that multiple dilutions will span the range of 0.1 – 300 ng/ml. Pipette 100 μ L of samples or standards into wells in the plate. Leave several wells empty for background binding determination.
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 50 μ l to 5 ml of 1x Dilution Buffer.
6. Wash plate by emptying contents and adding 250 μ l 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 μ l of Reporting Antibody into each well. Care should be taken to match the reporting antibody to the corresponding capturing antibody (e.g. C1 area adds R1 reporting antibody). Cover plate with the plate seal and incubate plate **45 minutes** at room temperature.
8. During the above incubation, dilute the 4 μ g/ml Streptavidin-HRP conjugate to 0.1 μ g/ml by adding 375 μ l to 15 ml of 1x Dilution Buffer.
9. Wash plate by emptying contents and adding 250 μ l 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 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 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 μ l 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 μ l 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.



PLATE TEMPLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
B	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
C	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
D	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
E	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
F	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
G	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample
H	C1-R1 STD	C1-R1 STD	C1-R1 Sample	C2-R2 STD	C2-R2 STD	C2-R2 Sample	C3-R3 STD	C3-R3 STD	C3-R3 Sample	C4-R4 STD	C4-R4 STD	C4-R4 Sample

