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HEK293T HCP ELISA KIT

Complete kit for the determination of HEK293T host cell protein contamination bulk products expressed in HEK293T expression systems.

INCLUDED

- Coated 96-Well Strip Plate
- HEK293T Protein Standard
- 1x Dilution Buffer
- 10x PBS-T
- Reporting Antibody
- Streptavidin-HRP Conjugate
- TMB Substrate
- Stop Solution
- Plate Sealer

PRODUCT MANUAL HCP-020

HEK293T HOST CELL PROTEIN ELISA KIT

ASSAY PRINCIPLE

The HEK293T Host Cell Protein (HCP) ELISA kit is designed to quantitatively measure HCP contamination in bulk products expressed in HEK293T expression systems. A vial of concentrated HEK293T protein is provided to generate a standard curve for the assay. HEK293T standards or unknown samples are pipetted into the provided 96-well strip plate, which has been pre-coated with anti-HEK293T HCP antibodies to capture HEK293T proteins from biologics samples. Following an incubation to allow capture of the HEK293T protein by the antibodies on the plate, a second anti-HEK293T HCP antibody, conjugated with biotin, is added and incubated to allow it to bind to the captured HEK293T 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 HEK293T protein standards and used to calculate the concentration of HEK293T 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.

Note: reporting antibody must be prepared 3 hours prior to use.

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-HEK293T HCP IgG. Can be used as individual strips.

HEK293T Protein Standard (1000 µg/ml, 50 µl)
Concentrated HEK293T proteins sufficient for generating a standard curve from 27 µg/ml to 37 ng/ml.

1x Dilution Buffer (75 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.

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

Reporting Antibody (175 µl/tube)
A biotin labeled rabbit polyclonal antibody specific for HEK293T cell proteins. **Three hours** prior to the assay, dilute 150 µl into 15 ml of 1x Dilution Buffer.

Streptavidin-HRP Conjugate (40 µg/ml, 175 µl/tube)
A Streptavidin–Horse Radish Peroxidase conjugate in a stabilizing solution. Immediately prior to the assay, dilute 150 µl into 15 ml of 1x Dilution Buffer to give a 0.4 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

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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 to 1x-strength with milliQ water. The 25 ml of 10x PBS-T should be diluted to 250 ml with 225 ml milliQ water.

2. Prepare the reporting antibody by adding 150 µl of reporting antibody to 15 ml of 1x Dilution Buffer. **Incubate 1.5 hours at room temperature before proceeding to step 3.** Continue to incubate for an additional 1.5 hours concurrent with step 5**, for a total incubation time of 3 hours.**

3. Prepare the HCP standards by numbering eight 1.5 ml tubes, and add 1168 µl of 1x Dilution Buffer to tube 1 and 800 µl of 1x Dilution Buffer to tubes 2-8. Cap the eighth tube, this will be the blank (0 ng/ml HCP). To tube one add 32 µl of the provided 1000 µg/ml HCP stock and mix well, this will be the 27 µg/ml standard. Then serially dilute 400 µl 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.

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

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

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 40 µg/ml Streptavidin-HRP conjugate to 0.4 µg/ml by adding 150 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 25 minutes will be sufficient; incubating longer may increase the background.

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

15. 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 HEK293T protein standards and used to calculate the concentration of HEK293T proteins in the unknown samples, taking into account any unknown sample dilution made.