Product Manual

HEK293T Host Cell Protein ELISA Kit

Catalog #: ENZ-KIT162

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

For the latest product information, including support documentation, visit us online:
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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 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 of the HEK293T protein by the antibodies on the plate, a second anti-HEK293T 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.**

SAFETY WARNINGS & PRECAUTIONS

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Handle with care

- 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 2.

Avoid freeze / thaw cycles

- The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

MATERIALS SUPPLIED

Entire kit must be stored at +4°C.

1. **Coated Clear 96 Well Plates**

   A clear plastic microtiter plate with break apart strips coated with rabbit anti-HEK293T HCP IgG.

2. **HEK293T Protein Standard (750 µg/mL, 46 µL/tube)**

   Concentrated HEK293T proteins sufficient for generating a standard curve from 27 µg/mL – 37 ng/mL.

3. **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.

4. **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 ultrapure water to achieve 1X PBS-T.

5. **Reporting antibody (175 µL/tube)**

   A biotin-labeled rabbit polyclonal antibody specific for HEK293T host cell proteins. **Three hours** prior to the assay, dilute 150 µL into 15 mL of 1X Dilution Buffer.

6. **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.

7. **TMB Substrate (15 mL)**

   Use directly without dilution.

8. **Stop Solution (15 mL)**

   A 1M solution of sulfuric acid. CAUSTIC. Use directly without dilution. This solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

9. **Plate Sealer (1)**

OTHER MATERIALS NEEDED

1. Distilled or deionized water (ultrapure water recommended)
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.

ASSAY PROTOCOL

1. Dilute the 10x PBS-T to 1X-strength with ultrapure water. The 25 mL of 10X PBS-T should be diluted to 250 mL with 225 mL ultrapure 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 1,157 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 43 µL of the provided 750 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 10-15 minutes 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 HEK293T protein standards and used to calculate the concentration of HEK293T proteins in the unknown samples, taking into account any unknown sample dilution made.

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:

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	Mean OD
NSB (0ng/mL HCP)	0.185
37.04 ng/mL HCP	0.182
111.1 ng/mL HCP	0.193
333.3 ng/mL HCP	0.228
1000 ng/mL HCP	0.309
3000ng/mL HCP	0.564
9000ng/mL HCP	1.100
27000ng/mL HCP	2.092

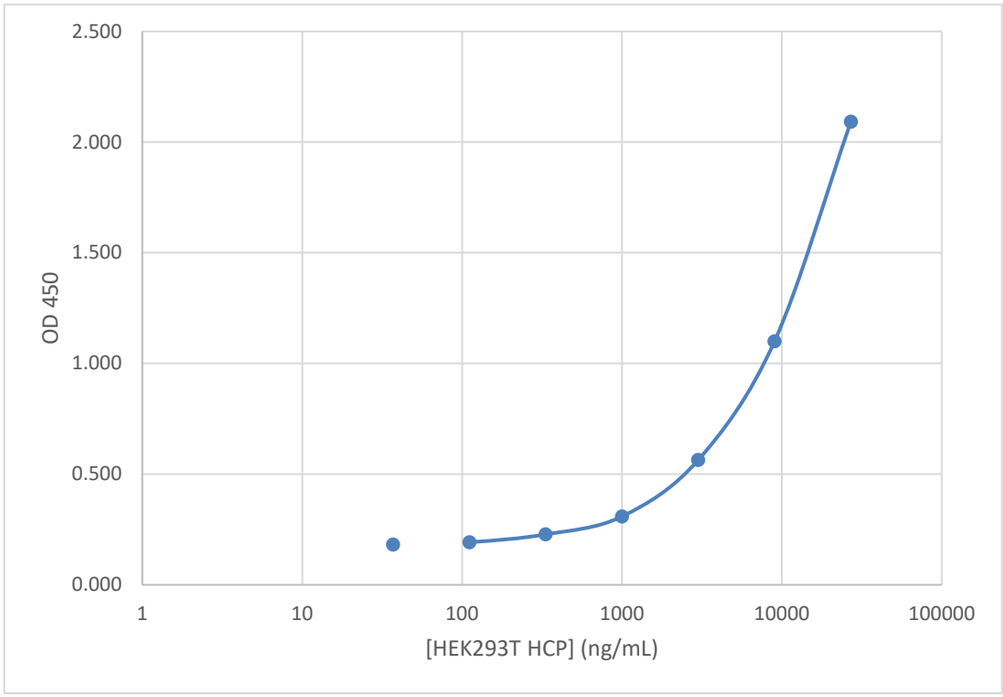

Plate Template

	A	B	C	D	E	F	G	H
1								
2								
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5								
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10								
11								
12								

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