Instructions for Use of 293T HCP ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG-HCP001

Introduction

This product is applicable for assay of 293T HCP in test samples by double-antibody sandwich method. The standard and test samples are added to reaction wells on microplate pre-coated with 293T antibody for incubation. The present 293T HCP quantitatively binds to the antibodies in the microplate, while unbound substances are removed after plate washing. Add Anti-293T HCP -Biotin and Streptavidin HRP successively to form an antibody - antigen - biotinylated antibody - enzyme-labeled avidin complex. The protein content in the test sample can be indicated by the color development degree of TMB. Please read the instructions for use carefully and check the components of the kit before use.

Assay range: 37-27000 ng/mL
Limit of quantification: 37 ng/mL
Precision: CV%≤10%, RE%≤±15%

Specification

96 T

Usage

This kit is designed for the quantitative detection of HCP (host cell protein) content in biopharmaceuticals expressed on 293T cells by using a double-antibody sandwich method, and can be used to detect all components of HCP (host cell protein) in 293T cell.

Kit components

Components	Specification	Preparation
293T HCP Coated Plate	8 wells × 12 strips × 1 piece	Ready-to-use
Anti-293T HCP-Biotin (detection antibody)	150 μL × 1 vial	1: 99, dilute with Diluent Buffer
Streptavidin HRP (enzyme conjugate)	375 μL × 3 vials	1: 9, dilute with Diluent Buffer
293T HCP Standard (standard)	600 μL × 1 vial (81 μg/mL)	Operate as per the recommended dilution procedure
Diluent Buffer	1 g× 1 vial	Use 1xPBS-T for current use（1g/100ml）
10× PBS-T Wash Buffer	50 mL× 1 bottle	1: 9, dilute with deionized water
TMB Substrate	15 mL× 1 bottle	Ready-to-use
Stop Solution	15 mL× 1 bottle	Ready-to-use
Plate Sealer	1 piece	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Notes: The product should be stored at 2 ~ 8℃ while being protected from light; the shelf life is 8 months.

Apparatus and materials to be prepared by the user:

- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized water
- Unused filter paper
- Vortex shaker

Reagent preparation

1. Temperature equilibration: Transfer reagents to be used to room temperature (18 ~ 25℃) environment and equilibrate the temperature for 30 minutes.

2. Preparation:

① 1× PBS-T Wash Buffer: Calculate the volume of working buffer required, measure an appropriate amount of 10× PBS-T Wash Buffer, dilute with deionized water at 1:9, and mix well for later use.

② Working solution of Diluent Buffer: Take an appropriate amount of diluent dry powder according to the experimental requirements, and dissolve it with 1x PBS-T at a ratio of 1g/100mL. It can also be diluted and packaged at once, and stored at -20 degrees Celsius. Avoid repeated freezing and thawing of diluent.

③ Detection antibody working buffer: Calculate the volume of working buffer required, measure an appropriate amount of biotinylated antibody, dilute with Diluent Buffer at 1:99, and mix well for later use.

④ Enzyme conjugate working buffer: Calculate the volume of working buffer required, measure an appropriate amount of enzyme conjugate, dilute with Diluent Buffer at 1:9, and mix well for later use.

⑤ The standard and test samples should be diluted with the Diluent Buffer.

3. Dilution of standard:

Vial No.	Standard solution concentration (μg/mL)	Standard solution volume (μL)	Diluent Buffer volume (μL)	Total volume (μL)	Final concentration (μg/mL)	Remaining volume (μL)
8	81000	110	220	330	27000	220
7	27000	110	220	330	9000	220
6	9000	110	220	330	3000	220
5	3000	110	220	330	1000	220
4	1000	110	220	330	330	220
3	330	110	220	330	110	220
2	110	110	220	330	37	330
1	/	/	220	220	0	220

Operation procedures

1. Mix all reagents well before use to avoid bubbles.
2. Confirm the number of stripe plates required based on the number of experimental wells. Put remaining strip plates back to aluminum foil bags with desiccants and seal the bag.
3. Loading: Add standard, sample, and negative control into respective wells at 100 μL/well. Seal the plate with a plate sealer,

and allow to react for 1.5 h at room temperature.

4. Plate washing: Discard the liquid in each well, and fill the wells with 1× PBS-T Wash Buffer (250 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on the filter paper after each washing.

5. Addition of biotinylated detection antibody working buffer: Add 100 µL of biotinylated detection antibody working buffer to each well, and react for 45 minutes at room temperature after sealing the plate with a plate sealer.

6. Plate washing: Discard the liquid in each well, and fill the wells with 1× PBS-T Wash Buffer (250 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.

7. Addition of enzyme conjugate working buffer: Add 100 µL of enzyme conjugate working buffer to each well, and react for 30 minutes at room temperature after sealing the plate with a plate sealer.

8. Plate washing: Discard the liquid in each well, and fill the wells with 1× PBS-T Wash Buffer (250 µL/well). Stand for 2 minutes and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.

9. Color development: Add 100 µL of TMB Substrate to each well, gently shake to mix well, seal the plate with a plate sealer, and place the plate at 25℃ for 15 minutes for color development reaction.

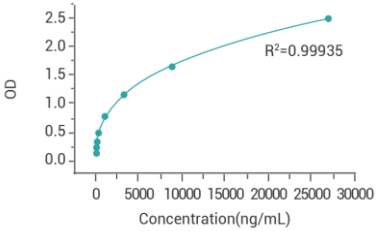
10. Assay: Add 100 µL of Stop Solution to each well and gently shake to mix well. Measure the optical density (OD) value of each well with a microplate reader at a primary wavelength of 450 nm.

Results process

1. The 4-parameter fitting method is recommended for the linear fitting and calculation of the product.
OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail):

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
27000	2.507	2.494	2.501
9000	1.73	1.733	1.732
3000	1.126	1.111	1.119
1000	0.773	0.77	0.772
333	0.496	0.475	0.486
111	0.289	0.285	0.287
37	0.205	0.195	0.200
0	0.140	0.137	0.139

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below)



Limitations of the assay method

The reagent is for assay of 293T HCP in test samples only.

Precautions

1. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
2. Before using the pre-coated strip plates, please equilibrate to room temperature before opening the secondary packaging. Strip plates not used in the experiment should be put back to the package immediately, and the package should be sealed tight. The plates may be stored for 1 month at 4℃. Other unused reagents should be packaged or covered.
3. The volumes of standard, biotin, and enzyme conjugate are all very small. Please perform rapid centrifugation before use to let liquid on the tube wall or cap gather at tube bottom.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each reagent in the kit before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well to absorb the liquid.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. The kit is for single use. Please use within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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