TECHNICAL MANUAL

HEK293T HCP ELISA Detection Kit (AEGE00003)

- **SKU CODE:** AEGE00003
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 07/2025

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Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Assay Range:

37-27000 ng/mL

Limit of quantification:

37 ng/mL

Detection Method:

Sandwich, Double Antibody

Sample Type:

HEK293T cell cultures

Precision:

CV% ≤10%, RE% ≤±15%

Storage:

4°C, 12 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C . Validity for 12 months. Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie 293T HCP ELISA Kit is designed for the quantitative detection of 293T host cell proteins (HCP) in test samples using a double-antibody sandwich ELISA format. This kit is designed for use in biopharmaceutical process development and quality control, where monitoring and quantifying residual HCPs is essential to ensure product purity, safety, and regulatory compliance. Typical applications include the analysis of in-process samples, purification intermediates, and final drug substance to verify the effective removal of HCP contaminants during upstream and downstream processing.

Each microtiter plate is pre-coated with a 293T-specific capture antibody. Standards and test samples are added to the wells incubated. During incubation, any 293T HCP present in the sample binds to the immobilized antibody. Unbound components are then removed through washing.

A biotin-labelled Anti-293T HCP detection antibody and Streptavidin-HRP are added sequentially to form an antibody–antigen–biotinylated antibody–enzyme complex. The presence of 293T HCP is determined by the intensity of the colour developed following the addition of TMB substrate.

4. Kit Contents

No	Component Name	Specification	Preparation
1	293T HCP Coated Plate	8 wells × 12 strips × 1 piece	Ready-to-use
2	Anti-293T HCP-Biotin (detection antibody)	150 µL × 1 vial	1: 99, dilute with Diluent Buffer
3	Streptavidin HRP (enzyme conjugate)	375 µL × 3 vials	1: 9, dilute with Diluent Buffer
4	293T HCP Standard (standard)	600 µL × 1 vial (81 µg/mL)	Operate as per the recommended dilution procedure
5	Diluent Buffer	1 g× 1 vial	Use 1xPBS-T for current use (1g/100 ml)
6	10× PBS-T Wash Buffer	50 mL× 1 bottle	1: 9, dilute with deionized water
7	TMB Substrate	15 mL× 1 bottle	Ready-to-use
8	Stop Solution	15 mL× 1 bottle	Ready-to-use
9	Plate Sealer	1 piece	Ready-to-use
10	Technical Manual	1 copy	

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. Store all reagents according to the instructions on the product label. Before use, allow all reagents to equilibrate to room temperature.
2. Before opening the secondary packaging, bring the pre-coated strip plates to room temperature. Return any unused strips immediately to the original packaging and reseal tightly. Store unused plates at 4°C for up to one month. All other unused reagents should be properly sealed or covered.
3. The volumes of the standard, biotinylated antibody, and enzyme conjugate are small. Perform a quick centrifugation prior to use to ensure that any liquid adhering to the tube walls or caps collects at the bottom.
4. Always use disposable pipette tips during the assay to prevent cross-contamination.
5. Inspect all kit components before use. To ensure accurate results, mix thoroughly when preparing dilutions, loading samples, or adding stop solution.
6. During the washing steps, after removing Wash Buffer, tap the plate dry on clean absorbent paper until no residual droplets or watermarks are visible. Do not insert tissue directly into the wells.
7. The TMB substrate is photosensitive, protect it from prolonged light exposure. Avoid contact with metal surfaces, as this may interfere with the reaction.
8. This kit is intended for single use and should be used within its stated shelf life.

6. Sample Preparation

Due to the inherent variability of biological samples and the specific requirements of individual assays, users are advised to optimize protocols in accordance with their own experimental conditions. Samples may be tested directly with this ELISA or diluted as necessary, based on experimental objectives and the physicochemical characteristics of the sample matrix.

Note: *For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

7. Reagent Preparation

1. **Temperature equilibration**: Before use, allow all reagents to equilibrate at room temperature (18–25°C) for at least 30 minutes.
2. **Reagent Preparation**:
    a. **1x PBS-T Wash Buffer:** Calculate the total volume needed. Dilute the 10xPBS-T Wash Buffer with deionized water at a ratio of 1:9. Mix thoroughly before use.
    b. **Diluent Buffer Working Solution:** Weigh the appropriate amount of diluent powder based on your experimental needs and dissolve it in 1x PBS-T at a ratio of 1g per 100 mL. The diluent can be prepared in bulk, aliquoted, and stored at –20°C. Avoid repeated freeze-thaw cycles.
    c. **Biotinylated Detection Antibody Working Solution:** Determine the required volume. Dilute the biotinylated antibody 1:99 with the prepared Diluent Buffer. Mix well before use.
    d. **Enzyme Conjugate Working Solution:** Calculate the necessary volume. Dilute the enzyme conjugate 1:9 with the Diluent Buffer and mix thoroughly.
    e. **Sample and Standard Dilution:** Dilute all standards and test samples using the prepared Diluent Buffer according to the assay requirements.

3. **Standard Dilution:**

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

8. Assay Procedure

1. **Reagent Mixing:** Thoroughly mix all reagents before use to ensure consistency and minimize bubble formation.
2. **Plate Preparation:** Determine the number of wells required based on your assay setup. Return any unused strip wells to the foil pouch with desiccant and reseal immediately.
3. **Sample Loading**: Add 100 µL of standard, sample, or negative control to the designated wells. Seal the plate with a plate sealer and incubate at room temperature for 1.5 hours.
4. **Plate Washing:** Discard the liquid from each well and add 250 µL of 1x PBS-T Wash Buffer. Let stand for 30 seconds, then discard the liquid. Repeat this wash step 3 times. After each wash, gently tap the plate dry on absorbent paper.
5. **Addition of Biotinylated Detection Antibody:** Add 100 µL of biotinylated detection antibody working solution to each well. Seal the plate and incubate at room temperature for 45 minutes.
6. **Plate Washing:** Repeat the same washing procedure as described in Step 4.
7. Addition of Enzyme Conjugate: Add 100 µL of enzyme conjugate working solution to each well. Seal the plate and incubate at room temperature for 30 minutes.
8. **Plate Washing:** Repeat the same washing procedure, this time allowing the wash buffer to stand in the wells for 2 minutes during each of the 3 wash cycles.
9. **Colour Development:** Add 100 µL of TMB Substrate to each well. Gently shake to mix, seal the plate, and incubate at 25°C for 15 minutes in the dark for color development.
10. **Assay Measurement:** Add 100 µL of Stop Solution to each well and mix gently. Measure the optical density (OD) of each well at 450 nm using a microplate reader.

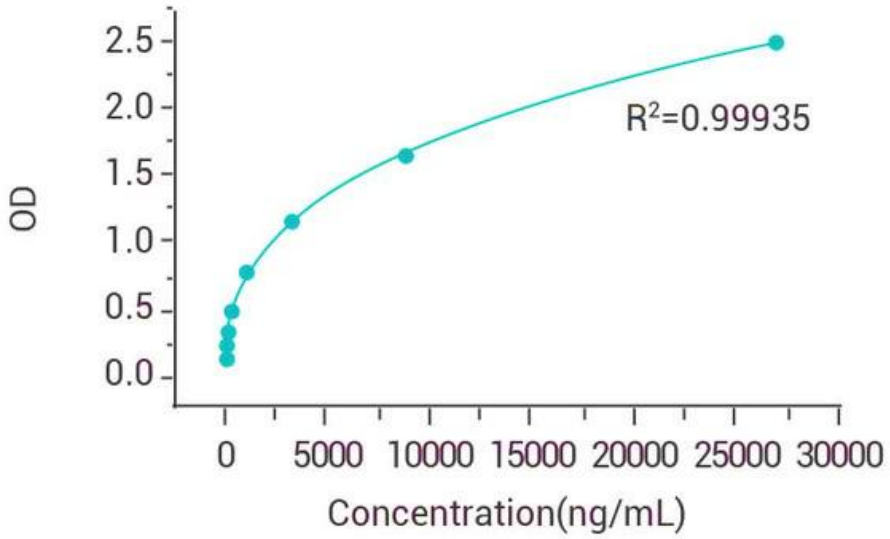
9. Data Analysis

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).

Std 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

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).



10. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.