NEED HELP?

# User Manual

Disclaimer: Products are intended for research use only

SHENTEK Biofargo

www.biofargo.com
804-529-2296
contact@biofargo.com

# HEK293 HCP ELISA Kit

# （One-step ELISA）

# User Guide

**PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT**

Product No.: 1301311Z01
Version: A/0
For Research Use Only

Biofargo, Inc.

## ■ Product Name

HEK293 HCP ELISA Kit (One-step ELISA)

## ■ Package

96 tests/Kit

## ■ Intended Use

This kit is intended for use in determining the presence of host cell proteins (HCPs) in products manufactured by expression in HEK293 derived host cells, including but not limited to recombinant proteins, cell and gene therapy products.

The kit is for RESEARCH USE ONLY and not intended for clinical use.

## ■ Product Description

This kit utilizes a solid-phase Enzyme-linked Immunosorbent Assay (ELISA) with a double-antibody sandwich technique to detect residual host cell proteins (HCPs) from HEK293 cells in the sample. A polyclonal antibody specific to HEK293 HCPs was employed in the assay to capture any remaining HCPs in the sample. Both the Calibration Standard (or test sample) and the HRP (Horseradish Peroxidase) labeled anti-HEK293 HCP antibody were simultaneously added to the microtiter plate, which coated with the affinity purified capture antibody and followed by incubation and washing. Then TMB (3,3',5,5' -tetramethylbenzidine) substrate was added into reaction, HRP catalyzed the oxidation of TMB by $H_2O_2$ to produce a blue product (maximum absorption peak at 655 nm). Then the stop solution was added to terminate the enzymatic reaction, resulting in a yellow colored product (maximum absorption peak at 450nm). The absorbance values at 450nm wavelength were positively correlated with the HCPs concentration in the Calibration Standard and the sample. The concentration of HCPs in the sample can be calculated using the dose-response curve.

No special treatment is required for the test sample, and its suitability could be verified by the appropriate dilution ratios with the kit.

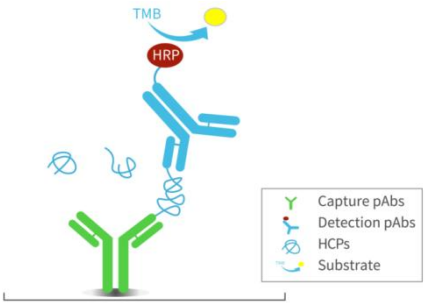



Figure 1. Schematic diagram

## ■ Kit Contents

Table 1. Kit Components

| Reagent | Part No. | Quantity | Note |
|---|---|---|---|
| HEK293 HCP Calibration Standard | PNB008 | 3 bottles | Lyophilized powder. Dissolve it with 500 µL Reconstitution Solution and let it stand for about 5 minutes. The solution should be clear and transparent. Please refer to the bottle label for details. |
| Anti-HEK293 HCP Microtiter Strips | PNA027 | 8 well × 12 strips | Strips pre-coated with sheep anti-HEK293 HCP affinity antibody in a vacuumed bag with desiccant. Seal and store immediately after use. |
| Reconstitution Solution | PNC002 | 2 × 1.5 mL | Only used to dissolve HEK293 HCP Calibration Standard. |
| Diluent | PNE015 | 2 × 25 mL | For dilution of Calibration Standard, Anti-HEK293:HRP (100×) and samples. |
| Wash Buffer Concentrate (10×) | PNF001 | 1 × 25 mL | Dilute 10 times with freshly prepared ultra-pure water for plate washing. |
| Anti-HEK293:HRP (100×) | PNN017 | 1 × 120 µL | Affinity purified sheep antibody conjugated to HRP. Dilute 100 times with Diluent before use. |
| TMB Substrate | PND004 | 1 × 12 mL | Equilibrate to room temperature (RT) for 20 minutes before use. Keep away from light and sealed. |
| Stop Solution | PNI002 | 1 × 6 mL | Avoid direct contact with eyes, skin, and clothing. |
| Sealing Film | PNK001 | 3 pieces | Cover the strips with it during incubation to prevent contamination and liquid evaporation. |

Note: Room temperature refers to 25±3℃.

## ■ Storage Conditions

Store the kit at 2-8℃. Please check the expiration date on the labels. The opened components should be stored as follows.

Table 2. Recommended storage conditions for opened components

| Component | Stability |
|---|---|
| Anti-HEK293 HCP Microtiter Strips | Store in the vacuumed bag with desiccant at 2-8℃ for up to 60 days. |
| Reconstituted HEK293 HCP Calibration Standard | Store at -20℃ and no more than 3 freeze-thaw cycles. |

## ■ Materials Required But Not Supplied in the Kit

- Sterile centrifuge tubes for dilution
- Absorbent paper for plate drying
- Pipette Tips: 1000 μL, 100 μL and 10 μL
- Multi-channel reagent reservoirs (50 mL)

## ■ Equipment

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm to 650 nm.
- Single or multi-channel pipettes: 1000 μL, 100 μL and 10 μL
- Microplate thermoshaker
- Incubator (optional)
- Plate washer (optional)

## ■ Workflow

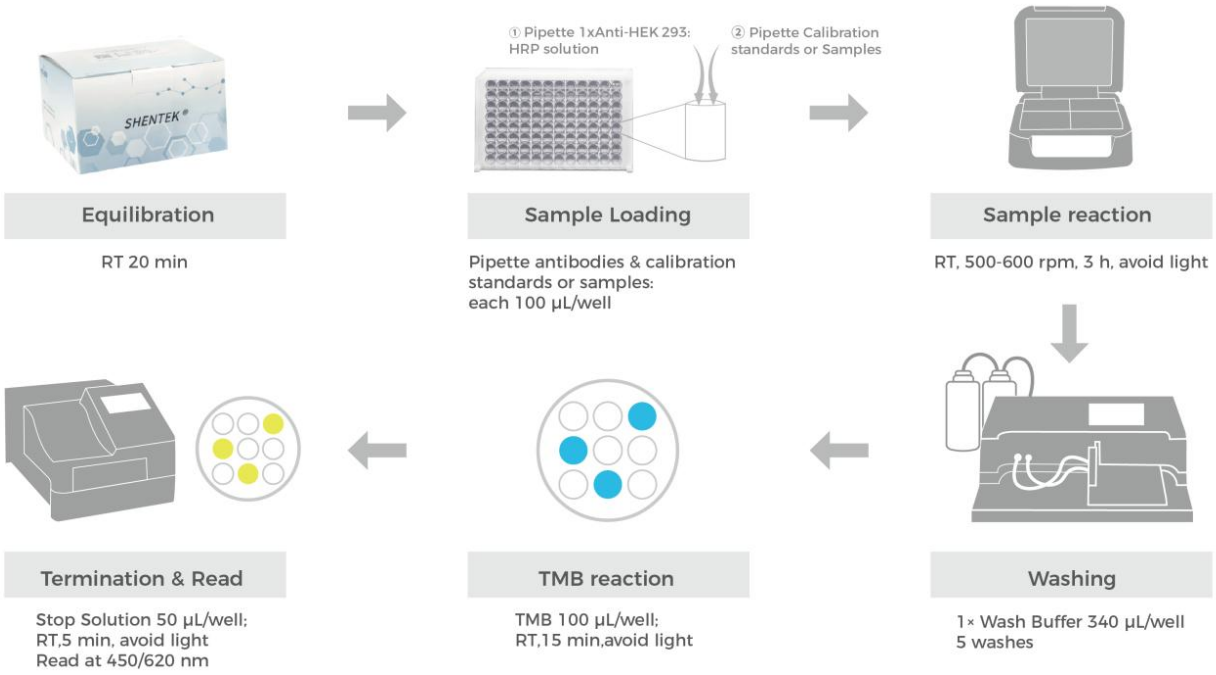



Figure 2. Procedure Flowchart

# 1. Preparation

## (1) Equilibration

- Allow the kit to equilibrate at room temperature for 20 minutes before use. Return to 2-8℃ after use.
- Take the appropriate amount of strips to a strip holder according to the experiment design and store the remaining strips in the bag with desiccant at 2-8℃.

## (2) Preparation of Reagents

- HEK293 HCP Calibration Standard solution: Pipette 500 µL of Reconstitution Solution into the bottle containing HEK293 HCP Calibration Standard. Gently invert to mix and let it stand for 5 minutes. Save the remaining solution under the recommended condition.

  Note: Do not use other volumes of Reconstitution Solution to dissolve the Calibration Standard.
- 1×Wash Buffer: Dilute 1 volume of Wash Buffer Concentrate (10×) with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate (10×) to 225 mL of ultra-pure water to make 250 mL of 1×Wash Buffer. Mix

well before use.

Note: If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, heat at 37 ℃ until it clears.

- 1×Anti-HEK293:HRP: Prepare the 1×Anti-HEK293:HRP by diluting the Anti-HEK293:HRP (100×) with Diluent in a sterile centrifuge tube. Gently mix the solution and use it immediately.

## (3) Preparation of Calibration Standard Solutions

- According to Fig 3 and Table 3, prepare HEK293 HCP Calibration Standard Solutions.

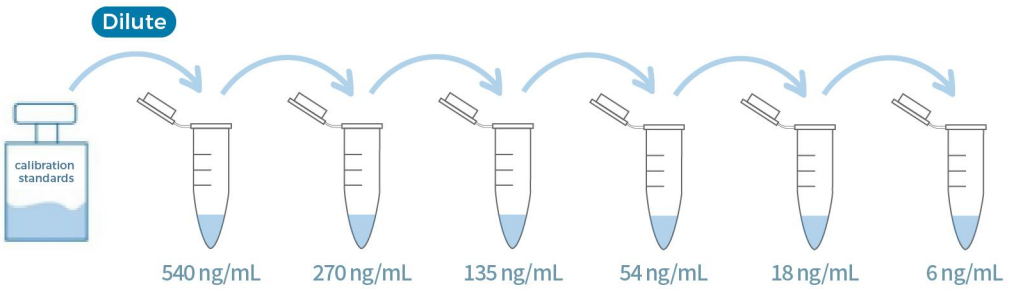



Figure 3. Graphic scheme of HEK 293 HCP Calibration Standard Solutions

Table 3. Preparation of HEK 293 HCP Calibration Standard Solutions

| Serial Dilution Tube | Dilution procedure | Conc. (ng/mL) |
|---|---|---|
| ST1 | Dilute the reconstituted HEK293 HCP Calibration Standard to ST1 | 540 |
| ST2 | 500 µL ST1 + 500 µL Diluent | 270 |
| ST3 | 500 µL ST2 + 500 µL Diluent | 135 |
| ST4 | 360 µL ST3 + 540 µL Diluent | 54 |
| ST5 | 300 µL ST4 + 600 µL Diluent | 18 |
| ST6 | 300 µL ST5 + 600 µL Diluent | 6 |
| NCS | Diluent | 0 |

## (4) Sample Preparation

- Test samples: In-process samples, drug substance and drug product. Samples should be clear and transparent, and insoluble substances need to be removed from samples through centrifugation or filtration.
- Conduct sample stability studies to prevent degradation or denaturation during the experiment. Long-term storage at -70°C is recommended to avoid degradation,

and avoid repeated freeze-thaw cycles.

- Dilute the samples with a suitable diluent to achieve a concentration of host cell proteins (HCPs) within the quantification range of the calibration curve.
- Recommend to verify sample suitability by determining the appropriate sample dilution factor before the initial test and facilitating the subsequent routine testing.

Note: Please contact us for support of validation protocol.

## 2. Assay Experiment

### (1) Sample Loading

- Pipette 100 μL of 1×Anti-HEK293:HRP into each designated well according to the experimental design.
- Pipette 100 μL of Calibration Standard, NCS (Diluent) and samples into the corresponding wells as indicated earlier. Avoid foaming bubbles during pipetting. It is recommended to prepare 2-3 parallels for each concentration.
- Seal the plate and incubate on microplate thermoshaker at 500-600 rpm for 3 hours at room temperature and protect from light.

Table 4. Example of 96-well plate layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NCS | NCS | NCS | | S1 | S1 | S1 | | | | | |
| B | | | | | S2 | S2 | S2 | | | | | |
| C | ST6 | ST6 | ST6 | | S3 | S3 | S3 | | | | | |
| D | ST5 | ST5 | ST5 | | S1+SRC | S1+SRC | S1+SRC | | | | | |
| E | ST4 | ST4 | ST4 | | S2+SRC | S2+SRC | S2+SRC | | | | | |
| F | ST3 | ST3 | ST3 | | S3+SRC | S3+SRC | S3+SRC | | | | | |
| G | ST2 | ST2 | ST2 | | | | | | | | | |
| H | ST1 | ST1 | ST1 | | | | | | | | | |

✧ “ST1-ST6” means 6 concentration gradients, “NCS” means negative control, “S1-S3” means test samples , and “S1 SRC-S3 SRC” means the spiked recovery controls for each sample.

✧ The number of replicates and the inclusion of spiked samples can be determined based on the results of method validation.

### (2) Substrate Incubation

- Equilibrate the TMB Substrate for 20 minutes at room temperature.
- Wash the plate with 340 μL of 1× Wash Buffer per well. Wipe off any liquid from the bottom outside of the plate. Repeat washing for 5 times. Do not allow the wells to dry before adding the substrate.
- Add 100 μL of TMB Substrate into wells, and incubate at room temperature for 15 minutes, protect from light.

  Note : Do not use sealing film for this step.

### (3) Termination & Reading

- Add 50 μL of Stop Solution into each well.

  Note: The adding sequence should be the same as the adding sequence of the TMB Substrate . Suspend the tips while adding samples to prevent contact with the solution in the wells and minimize the risk of bubble formation.
- Incubate at room temperature for another 5 minutes, protect from light.

## 3. Calculation and Analysis

- The $OD_{450\text{-}620}$ value of each well should be calculated by subtracting their respective long wavelength, as of $OD_{620\ nm}$ in this case. If the microplate reader is not equipped with long wavelength measurement, this step can be omitted.
- The $OD_{450\text{-}620\text{-}N}$ value of calibration curve fitting points and samples should be calcualted by substracting the $OD_{450\text{-}620}$ of NCS, then take the average value of replicates.
- Perform a 4-parameter logistic regression model using the Calibration Standard concentration values and OD values to obtain the calibration curve equation. Substitute the average OD value of the sample into the equation to calculate the sample concentration, which should be multiplied by the dilution factor to obtain the actual sample concentration.
- The software for analysis of the standard curve could be the one that comes with the microplate reader. If not, it is recommended to use professional standard curve software such as Curve Expert, ELISA Calc, and so on.

- For samples with absorbance values exceeding the Calibration Standard ST1, the appropriate dilution should be performed before retesting. The HCP concentration in the sample is calculated from the test value multiplied by its corresponding dilution factor. If the spiked samples are simultaneously set at this dilution level and the recovery rate should meet the requirements of the corresponding regulations.

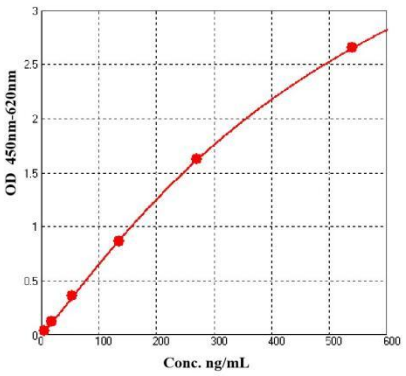
## ■ Limitations

- This product is intended for research use only but not for clinical applications.
- Specifically designed for detecting residual protein content in products manufactured from HEK293 host cells. A method validation is required before relying exclusively on this assay.
- Recommend sample pH between 6.5 and 8.5, and measurements may be compromised if the sample pH is too low or too high.

## ■ Assay Performance

- Linearity & Range: 6-540 ng/mL, $R^2 \geq 0.990$.
- LLOQ: 6 ng/mL.
- Specificity: No cross-reactivity with *E.coli* HCP, *P.pastoris* HCP, Sf9 HCP, CHO HCP and Vero HCP.
- Typical calibration curve and results:

| Calibration Standards (ng/mL) | Abs. At (450 nm-620 nm) | AVG |
|---|---|---|
| 540 | 2.7474 | 2.7308 |
| | 2.7167 | |
| | 2.7284 | |
| 270 | 1.7092 | 1.7061 |
| | 1.7139 | |
| | 1.6953 | |
| 135 | 0.9520 | 0.9437 |
| | 0.9373 | |
| | 0.9418 | |
| 54 | 0.4462 | 0.4398 |
| | 0.4309 | |
| | 0.4422 | |
| 18 | 0.2027 | 0.1998 |
| | 0.1969 | |
| | 0.1997 | |
| 6 | 0.1173 | 0.1165 |
| | 0.1206 | |
| | 0.1115 | |
| 0 | 0.0772 | 0.0793 |
| | 0.0805 | |
| | 0.0801 | |




4-PL: $Y = \frac{A-D}{1+(\frac{X}{C})^B} + D$

A = 5.71467
B = -1.13651
C = 615.49064
D = 0.01444
$R^2$ = 0.99993

## ■ Additional Information

- ✧ This kit is intended for use by qualified technicians only.
- ✧ Use sterile disposable tips, tubes and reservoirs, etc. separately. It is recommended to wipe with 75% ethanol before and after each use. Follow the specified pipetting procedure carefully.
- ✧ Users should validate the assay before testing their samples.
- ✧ Dilution should be gentle and thorough to avoid excessive foaming.
- ✧ Stop Solution is 1M HCl. Avoid direct contact with eyes, skin, and clothing.
- ✧ Do notmix the kit reagents from different lot numbers.
- ✧ Use fresh sterile water or ultra-pure water, and ensure the water temperature does not exceed 37℃.
- ✧ Seal or cover the microplate immediately after sample loading to avoid liquid evaporation.
- ✧ Avoid drying the wells before substrate incubation.
- ✧ Store unused microtiter strips in a sealed bag with desiccant to prevent contamination.
- ✧ Centrifuge Anti-HEK293:HRP(100×) before use avoid any loss of the reagent.
- ✧ Accurately pipetting or sampling for dilution of standards and samples, for example, minimum volume of 5 μL is recommended.
- ✧ HEK293 HCP Calibration Standard and 1×Anti-HEK293:HRP are recommended for single use due to instability issue. Prepare freshly before each experiment.
- ✧ TMB Substrate should be colorless. If not, discard it and contact us for assitance.
- ✧ Pipette carefully to avoid any bubbles, and gently shake the plate for thorough mixing. Sometimes air, resulting in bubbles, can be drawn into the pipette or dispensed into the wells. If this happens, bubbles can influence optical density values and results.
- ✧ Reading should be completed within 30 minutes after termination.
- ✧ Avoid the samples containing sodium azide ($NaN_3$), which will deactivate the HRP and lead to the underestimation of HCP levels.

## ■ Troubleshooting

| Problem | Possible Cause | Solution |
|---|---|---|
| High background signal (OD) | Cross-contamination of reagents, including distilled water | Freshly prepared prior to experiment. |
| | Cross-contamination of equipments, including pipettes and centrifuge | Clean the equipment with 75% ethanol before experiment. |
| | Environment contamination | Separate the working bench to avoid contamination. |
| | Insufficient washing | Increase the wash buffer volume or wash more times, and remove any remaining liquid before proceeding to the next step. |
| Abnormal values | Improper washing | Swiftly and completely shake off any excess liquid, and avoid reusing paper towels to minimize contamination. |
| | Improper sampling | Add the samples to the bottom of the wells using micropipettes, and avoid splashing to the neighboring wells. |
| | Plate sealing | Promptly cover the plate with the sealing film and remove it carefully to prevent splashing. |

*If any other difficulties, please contact us for technical support.*

## ■ References


- USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals
- USP <1225> Validation of Compendial Procedures
- EP <2.6.34> Host-Cell Protein Assays
- ICH Q2 (R2) Validation of Analytical Procedures
- ChP <9101> Guidance for Analytical Procedures Validation
- JP <G3-9-172> Host Cell Protein Assay


Effective date: 28 Aug. 2025

## Support & Contact


SHENTEK

Biofargo, Inc.
www.biofargo.com
Address: 1716 E Parham Rd Richmond, Va, 23228, USA
E-mail: contact@biofargo.com
Phone: 804-529-2296