



## 293T HCP ELISA Kit

293T HCP ELISA Kit is an Enzyme Immunoassay kit for the quantification of 293T HCP in biopharmaceuticals.

Catalog number: ARG83094

Package: 96 wells

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For research use only. Not for use in diagnostic procedures.

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## **INTRODUCTION**

293T HCP ELISA 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.

293T HCP ELISA Kit can be used to detect all components of HCP (host cell protein) in 293T cell.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for 293T has been pre-coated onto a 96 well microplate. 293T antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti-293T antibody is added and binds to 293T antigen captured by the first antibody. After washing away any unbound substances, a HRP Conjugate mouse anti-FITC antibody is added and incubation. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of 293T bound in the initial step. The color development is stopped by the addition of stop solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of 293T in the samples is then determined by comparing the O.D of samples to the standard curve.

## **MATERIALS PROVIDED & STORAGE INFORMATION**

Use the kit before expiration date.

<b>Component</b>	<b>Quantity</b>	<b>Storage information</b>
Antibody Coated Microplate	8 X 12 strips	4°C
Standard	600 µL (81 µg/mL)	-20°C
100X Antibody Conjugate	150 µL	-20°C
10X HRP- Streptavidin Solution	3 X 375 µL	-20°C
Diluent Buffer	1 g	4°C
10X Wash Buffer	50 mL	4°C
TMB Substrate	15 mL (ready to use)	4°C (protect from light)
STOP Solution	15 mL (ready to use)	4°C
Plate sealer	5 pieces	4°C

## **MATERIALS REQUIRED BUT NOT PROVIDED**

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer

## **TECHNICAL NOTES AND PRECAUTIONS**

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required

number of strips to room temperature (20-25°C).

- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- All reagents must be mixed without foaming and briefly spin down the all vials before use.
- If crystals are observed in the 20X PBST Wash Buffer or Diluent Buffer warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the mixed TMB Substrate. Do not expose the mixed TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- All reagents must be mixed without foaming before use.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.

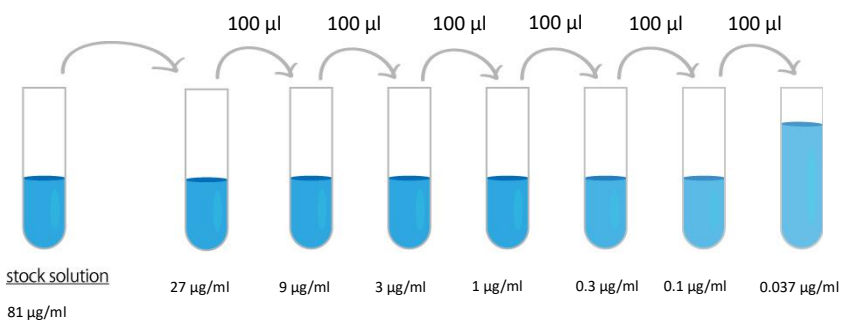
### REAGENT PREPARATION

- **1X Wash Buffer:** Dilute **10X Wash Buffer** into **distilled water** to yield **1X Wash Buffer**. The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C. Mix well before use.
- **Diluent Buffer:** Briefly spin down before opening. Add 100ml of **1X Wash Buffer** and vortex gently to completely dissolve contents to obtain working Diluent Buffer

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- **1X Antibody Conjugate:** It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute **100X** Antibody Conjugate Mixture concentrate into **Diluent Buffer** to yield 1X Antibody Conjugate.
- **1X HRP-Streptavidin Solution:** It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute **10X** HRP-Streptavidin Solution into **Diluent Buffer** to yield 1X HRP-Streptavidin Solution.
- **Standards:** The Diluent Buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **27 µg/ml, 9 µg/ml, 3 µg/ml, 1 µg/ml, 0.33 µg/ml, 0.11 µg/ml, 0.037 µg/ml**. DO NOT reuse the reconstituted standard.



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Dilute 293T HCP standard as according to the table below:

Standard	293T HCP Conc.	$\mu\text{l}$ of Diluent Buffer	$\mu\text{l}$ of standard
S7	27 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (stock)
S6	9 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (S7)
S5	3 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (S6)
S4	1 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (S5)
S3	0.33 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (S4)
S2	0.11 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (S3)
S1	0.037 $\mu\text{g}/\text{ml}$	200 $\mu\text{l}$	100 $\mu\text{l}$ (S2)
S0	0 ng/ml	300 $\mu\text{l}$	0

### ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use.

1. Add **100  $\mu\text{L}$**  of the **Standard** or **Samples** to the Antibody Coated microplate.
2. Cover the plate and incubate for **1.5 hours** at **room temperature**.
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1X Wash Buffer (250  $\mu\text{L}$ )** using a squirt bottle, manifold dispenser, or autowasher. Keep the wash buffer in the wells for 30-60 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100  $\mu\text{L}$**  of **1X Antibody Conjugate** to each wells.
5. Cover the plate and Incubate for **45 minutes** at **room temperature**.

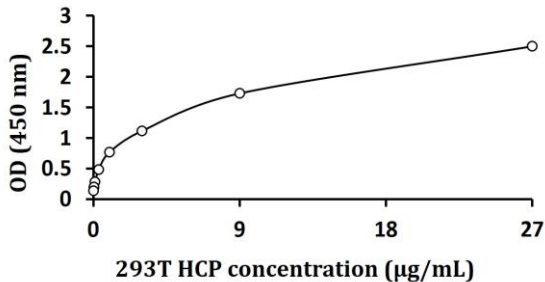
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- Aspirate each well and **wash as step 3**.
- Add **100  $\mu\text{L}$**  of **1X HRP-Streptavidin Solution** to each well. Cover the plate and incubate for **30 minutes** at **room temperature**.
- Aspirate each well and **wash as step 3**.
- Add **100  $\mu\text{L}$**  of **TMB Substrate** to each well. Cover and incubate for **10-20 minutes** at **room temperature** in the dark.
- Immediately Add **100  $\mu\text{L}$**  of **Stop Solution** to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
- Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 10 minutes** after adding the stop solution.

### EXAMPLE OF TYPICAL STANDARD VALUES

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



### CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of standards and samples.
- To obtain more accurate results, more dilution points can be used when



generating standard curves. 4 Parameter Logistics is the preferred method for the result calculation. Other data reduction functions may give slightly different results.

3. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (<https://www.arigobio.com/elisa-analysis>)
4. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.