E.coli HCP ELISA Kit

E.coli HCP ELISA Kit is an Enzyme Immunoassay kit for the quantification of *E.coli* host cell protein in biopharmaceuticals.

Catalog number: ARG83090

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Tel: +886-3-6221320

Fax: +886-3-5530266

Email: info@arigobio.com

INTRODUCTION

The *E.coli* HCP ELISA Kit is a kit used for the detection of Host Cell Protein (HCP) in Escherichia coli (*E.coli*). It is based on the principle of Enzyme-Linked Immunosorbent Assay (ELISA) and allows for accurate and sensitive quantification of HCP content in *E.coli.*

E.coli is a common gut bacterium and a frequently used host cell line in biotechnology and biopharmaceutical industries. The *E.coli* HCP ELISA Kit provides a reliable method for assessing the presence and concentration of HCP, which is essential for ensuring the quality and safety of biologics produced using E.coli expression systems.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. The antibody has been pre-coated onto a microtiter plate. Standard and samples are pipetted into the wells and any *E.coli* HCP present is bound by the immobilized specific antibodies. Then, added Antibody-Conjugate to each well and incubate. After washing away any unbound substances, a TMB substrate is added to the wells and color develops in proportion to the amount of target cytokines 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 O.D. 450 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Use the kit before expiration date.

Component	Quantity	Storage information
E.coli HCP Coated Microplate	8 X 12 strips	4°C
Standard	500 µL (2430 ng/mL)	4°C
Standard Diluent Buffer	30 ml (ready to use)	4°C
10X Antibody Conjugate	1.5 mL	4°C
Antibody Conjugate Diluent Buffer	15 ml (ready to use)	4°C
20X Wash Buffer	30 mL	4°C
TMB Substrate A	8 mL	4°C (protect from light)
TMB Substrate B	8 mL	4°C (protect from light)
STOP Solution	15 mL (ready to use)	4°C
Plate sealer	1 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

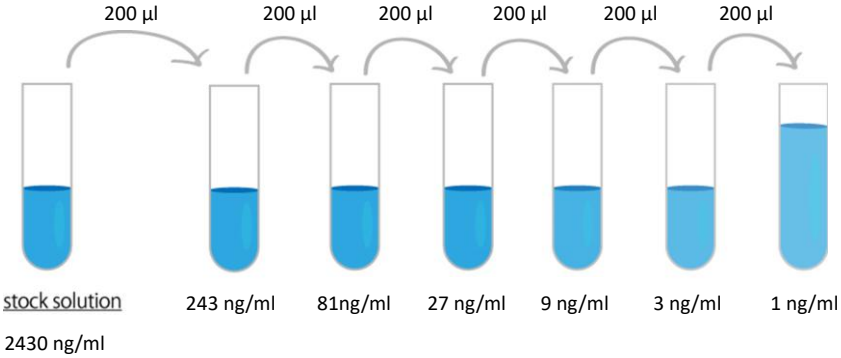
TECHNICAL NOTES AND PRECAUTIONS

- 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 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 20X 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.
- 1X Antibody Conjugate: It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute 10X Antibody Conjugate into Antibody Conjugate Diluent Buffer to yield 1X Antibody Conjugate.

- **Standards**: The Standard 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: **810 ng/ml**, **270 ng/ml**, **90 ng/ml**, **30 ng/ml**, **10 ng/ml**, **3.3 ng/ml**, **0 ng/ml**. DO NOT reuse the reconstituted standard.



Dilute *E.coli* HCP standard as according to the table below:

Standard	E.coli HCP Conc.	µl of Standard Diluent Buffer	µl of standard
S6	243 ng/ml	900 µl	100 µl (2430 ng/mL)
S5	81 ng/ml	400 µl	200 µl (S6)
S4	27 ng/ml	400 µl	200 µl (S5)
S3	9 ng/ml	400 µl	200 µl (S4)
S2	3 ng/ml	400 µl	200 µl (S3)
S1	1 ng/ml l	400 µl	200 µl (S2)
S0	0 ng/ml	400 µl	0 µl

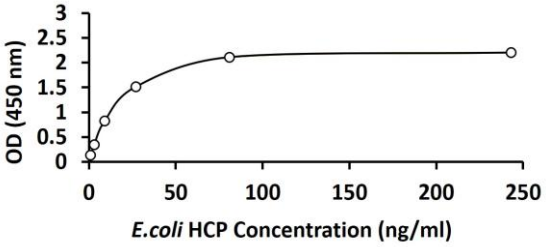
ASSAY PROCEDURE

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

1. Add **100 µL** of **Standard** or **Samples** to the Antibody Coated microplate.
2. Add **100 µL** of **1X Antibody Conjugate** to each well.
3. Cover the plate and incubate for **2 hours** at **room temperature**.
4. 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 µL)** 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.
5. Add **100 µL** of **TMB Substrate** to each well. Cover and incubate for **15-25 minutes** at **RT** in the dark.
6. Immediately Add **100 µL** of **Stop Solution** to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
7. 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

1. Calculate the average absorbance values for each set of standards and samples.
2. 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.