# E.coli|360-HCP ELISA

## *Developmental ELISA Kit for the Determination of E. coli-HCP in process-derived Samples*

**FOR IN VITRO USE ONLY**

## 1 INTENDED USE

The supplied E.coli|360-HCP ELISA kit is a sandwich ELISA to be performed in a microtest plate format. This assay is intended for the quantitative in-vitro measurement of *E. coli*-HCP (*Escherichia coli* Host Cell Protein) in process-related samples of recombinant production processes performed with respective *E. coli* cell line W3110.

This kit contains all necessary reagents to perform the HCP ELISA.

The assay covers a preliminary working range between 2 ng/mL to 100 ng/mL of *E. coli*-HCP.

## 2 REAGENTS AND MATERIALS PROVIDED IN THE KIT

| No. | Reagent | Details | Quantity |
|---|---|---|---|
| (1) | Microtest Plate (ready-to-use) | 96 well, pre-coated with affinity-purified anti-E.coli-HCP-IgG (polyclonal, Type A, C, D from goat, Type E from rabbit), dried and sealed in foil bag with desiccant ready-to-use microtest plate | 1x |
| (2) | Washing Buffer | Tris-based washing buffer for immunoassays, BlueCap Solutions S210, 10x concentrate | 1x 100 mL |
| (3) | Assay Buffer | Tris-based assay buffer for immunoassays, BlueCap Solutions S210, 10x concentrate | 1x 20 mL |
| (4) | E.coli-HCP Kit Standard (10 µg/mL) | Kit standard of *E. coli*-HCP stabilized Solution (Protein concentration was determined by Bradford Test – not BCA!) | 1x 0.1 mL |
| (5) | Detector Antibody (100x) | affinity purified, biotin-conjugated anti-E.coli-HCP- IgG (polyclonal, Type A, C, D from goat, Type E from rabbit) in stabilized solution, 100x concentrate | 1x 0.3 mL |
| (6) | Enzyme Conjugate (100x) | Streptavidin-conjugated Peroxidase in stabilized solution, 100x concentrate | 1x 0.3 mL |
| (7) | Substrate Solution (ready-to-use) | TMB One substrate solution, ready-to-use solution | 1x 15 mL |
| (8) | Stop Solution (ready-to-use) | 0.5 M sulfuric acid, ready-to-use solution | 1x 15 mL |

## 3 MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED WITH THE KIT

- Ultrapure water (at least double distilled quality) for dilution of the washing buffer (10x)
- Absorbent paper towels for removing residual liquid after microtest plate washing
- Suitable reaction tubes for preparing standards, controls and samples
- Suitable containers for washing buffer
- Suitable reagent reservoirs for effective multi-channel pipetting
- Lid for covering the microtest plates during the incubation steps
- Orbital microtest plate shaker (about 500 rpm) and vortex mixer
- Microtest plate washer (manual washing can alternatively be performed)
- Precision pipettes (adjustable volumes from 10 µL to 5000 µL) with suitable tips
- Multi-channel pipette (100 µL) with suitable tips
- Microplate reader for measuring optical density at 450 nm (reference wavelength of 620 - 690 nm)

## 4 STORAGE OF THE REAGENTS

The supplied reagents (1 to 3 and 5 to 8) should be kept refrigerated at 2 - 8°C and should be used before their expiration dates. The *E. coli*-HCP Kit Standard should be frozen in aliquots at <-60 ° C and thawed before usage.

## 5 TEST PRINCIPLE

The E.coli|360-HCP ELISA is based on a sandwich enzyme immunoassay in 96 well microtest plates (1x8F strip format). Samples which may contain *E. coli*-HCP impurities are incubated in microtest plate wells that are pre-coated with a polyclonal *E. coli*-HCP-specific capture antibody alongside with a calibration curve of different *E. coli*-HCP standard concentrations. After incubation and a plate washing step in which unbound components are removed, a specific biotin-conjugated *E. coli*-HCP detector antibody is added. After further washing steps, the bound detector antibody in turn reacts with the enzyme conjugate (streptavidin-conjugated peroxidase) as the tracer. After a final washing step, the color reaction is performed with tetramethylbenzidine (TMB) as enzyme substrate, resulting in a blue color. At the end of the substrate incubation time, the reaction is being stopped by the addition of sulfuric acid, converting the blue color to yellow and the optical density is measured photometrically at a wavelength of 450 nm (reference wavelength of 620-690 nm). The optical density in the wells is proportional to the *E. coli*-HCP concentration in the wells. The *E. coli*-HCP concentration of unknown samples can be calculated based on the corresponding *E. coli*-HCP calibration curve.

## 6 WARNINGS AND PRECAUTIONS

- This kit is intended for in-vitro research only and should only be used by qualified personnel.
- Before starting the assay, read the instruction leaflet carefully.
- Note lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
- Follow good laboratory practice and safety guidelines. Wear lab coats, disposable gloves and protective glasses when necessary.
- Some reagents of the kit contain Proclin®300 as preservative. These reagents may case eye and skin irritations and should be handled with care. In case of contact with eyes or skin, flush immediately with water.
- Store the substrate solution protected from light.
- The stop solution consists of 0.5 molar Sulfuric acid. This reagent is corrosive and may cause eye and skin irritations. It should be handled with care. In case of contact with eyes or skin, flush immediately with water.
- Remaining kit reagents and prepared solutions have to be treated as potentially hazardous waste according to national safety guidelines and regulations.

## 7 SAMPLE TREATMENT AND STORAGE

The HCP stability in process-derived samples has not been investigated yet. Therefore, the samples should be either stored refrigerated (2°-8°C) or frozen (equal to or below -20°C) depending on the temperature requirements of the recombinant product in the sample. In general, repeated freezing / thawing of samples should be avoided.

## 8 REAGENT PREPARATION

### 8.1 Preparation of the Washing Buffer (1x)

Dilute the washing buffer 10x (2) 1:10 with ultrapure water prior to use (e.g. 100 mL 10x concentrate with 900 mL ultrapure water). The washing buffer (1x) is stable at room temperature and can be used for two weeks upon day of preparation.

### 8.2 Preparation of the Assay Buffer (1x)

Dilute the assay buffer 10x (3) 1:10 with ultrapure water prior to use (e.g. 20 mL 10x concentrate with 180 mL ultrapure water). The assay buffer (1x) is stable at room temperature and can be used for two weeks upon day of preparation.

### 8.3 Preparation of the *E. coli*-HCP Standards

The HCP standard provided in this kit was prepared as a master standard from *E. coli* strain W3110 cell lysates. The protein concentration of the kit standard was adjusted based on the Bradford protein method.

The standard concentrations of the E.coli|360-HCP ELISA should be prepared from the respective *E. coli*-HCP standard (4) of the kit immediately before performing the assay.

8 ELISA standards (S1 to S8) are prepared in assay buffer (8.2) covering the assay working range (about 2 ng/mL to 100 ng/mL *E. coli*-HCP) according to the following dilution scheme. The standards should be used only at the day of preparation.

| Standard ID | Concentration [ng/mL] | Volume Antigen [µL] | | Volume Assay Buffer [µL] |
|---|---|---|---|---|
| | | | *of* | |
| Kit Standard | 10.000 | - | - | - |
| S1 | 100 | 10 | Kit Standard | 990 |
| S2 | 50 | 500 | S1 | 500 |
| S3 | 25 | 500 | S2 | 500 |
| S4 | 12.5 | 500 | S3 | 500 |
| S5 | 6.25 | 500 | S4 | 500 |
| S6 | 3.12 | 500 | S5 | 500 |
| S7 | 1.56 | 500 | S6 | 500 |
| S8 | - | - | - | 1000 |

### 8.4 Preparation of the Detector Antibody Working Solution (1x)

Dilute the detector antibody 100x solution (5) 1:100 with assay buffer (8.2). For one microtest plate 120 µL of the 100x concentrate are mixed with 12 mL assay buffer. This working solution should be used only at the day of preparation.

### 8.5 Preparation of the Enzyme Conjugate Working Solution (1x)

Dilute the enzyme conjugate 100x solution (6) 1:100 with assay buffer (8.2). For one microtest plate 120 µL of the 100x concentrate are mixed with 12 mL assay buffer. This working solution should be used only at the day of preparation.

## 9 SAMPLE PREPARATION

In case of the presence of aggregates, they should be removed by centrifugation to ensure proper assay performance. In general, all sample working dilutions should only be used on the day of preparation.

The samples should be diluted with assay buffer (8.2) prior to the assay. The minimum sample dilution should be 1:2. The optimum sample dilution has to be determined by the user for each sample type, depending on HCP content, taking the assay working range into account.

## 10 ASSAY PROCEDURE

All ELISA steps are performed at room temperature (18-26°C).

Allow all materials and reagents of the kit to reach room temperature before use.

Do not open the foil bag of the pre-coated microtest plate (1) before reaching room temperature. Remaining plate strips which were not used in the assay should be repacked to the bag with desiccant. Close the bag tightly for refrigerated storage.

During all incubation steps the plate should be covered with a lid (not provided with the kit) to prevent evaporation and contamination of solutions.

1. **Incubation with standards and samples:**

   Fill the plate with 100 µL/well of *E. coli*-HCP standards and assay controls as well as the diluted samples and incubate for 2 hours with continuous shaking. Standards, assay controls and samples should be analyzed at least in duplicates. Triplicates are recommended.

2. **Washing:**

   Remove the contents of the wells and wash the plate 4x with 250 µL/well of the washing buffer (1x). After washing, invert the plate and discard residual liquid by intensely tapping the plates on absorbent paper.

3. **Incubation with detector antibody:**

   Fill the plate with 100 µL/well of the detector antibody working solution (1x) and incubate 1.5 hours with continuous shaking.

4. **Washing:**

   Remove the contents of the wells and wash the plate 4x with 250 µL/well of the washing buffer (1x). After washing, invert the plate and discard residual liquid by intensely tapping the plates on absorbent paper.

5. **Incubation with enzyme conjugate:**

   Fill the plate with 100 µL/well of the enzyme conjugate working solution (1x) and incubate 20 minutes with continuous shaking.

6. **Washing:**

   Remove the contents of the wells and wash the plate 4x with 250 µL/well of the washing buffer (1x). After washing, invert the plate and discard residual liquid by intensely tapping the plates on absorbent paper.

7. **Incubation with substrate solution and stopping:**

   Add 100 µL/well of the substrate solution (7) and incubate the plate for 15 min with continuous shaking. If color development is too low after 15 minutes the substrate incubation can be extended up to 30 minutes.

   Stop the color reaction by adding directly 100 µL/well of the stop solution (8) into the plate resulting in a yellow colored product.

8. **Measurement:**

   Measure the optical density at 450 nm ($OD_{450}$) with a multichannel microplate reader and giving the raw data of the assays. A reference wavelength between 620 nm and 690 nm is recommended.

## 11 CALCULATIONS

For generating the standard curve and calculation of the assay controls and the samples, average the corresponding replicate $OD_{450}$ values of each standard.
Generate a standard curve with the aid of a suitable software, using preferably the nonlinear regression mode of the four- or five-parameter equation.
The concentration of unknown samples calculated from the calibration curve has to be multiplied by the dilution factor of the sample.

## 12 TROUBLESHOOTING

Possible reasons (explanations) for insufficient assay performance are listed below.

A. No reactivity throughout the whole plate

- *Omission of an incubation step or a reagent*
- *Use of reagents in a false order*
- *Inadequate preparation of ELISA components/ reagents*

B. Poor reactivity throughout the whole plate

- *Inadequate storage or preparation of ELISA components/ reagents*
- *Reagents were not allowed to reach room temperature before use*
- *Improper wavelength for measuring the optical density*

C. To high reactivity and/or assay background throughout the whole plate

- *Improper washing step(s)*
- *Inadequate storage or preparation of ELISA components/ reagents*
- *Overincubation of reagents, for example incubation with substrate solution before stopping*

D. Poor Intra-Assay Precision (too high CV of replicates)

- *Improper washing step(s)*
- *Insufficient mixing of standards, samples and assay control samples*
- *Inappropriate sample preparation (debris or aggregates in the samples disturb the assay performance by increasing the imprecision and should be removed by centrifugation).*