# Host Cell Protein – HCP (E.coli)

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# Host Cell Protein – HCP (E.coli)

# ELISA kit

# #A05034.96 wells

For research laboratory use only
Not for human diagnostic use

This assay was developed
& validated by Bertin Bioreagent

Fabriqué en France
Made in France

#A11034

Version: 0121

# Table of contents

**96 wells**
**Storage: +4°C**
**Expiry date: stated on the package**

This kit contains:

| Designation | Colour of cap | Item # | Quantity per kit | Form |
|---|---|---|---|---|
| 96 strip well Microtiter plate, pre-coated with anti-HCP polyclonal antibodies | Blister with zip | A08034.1 ea | 1 | - |
| Steptavidin HRP Tracer | Red | A22010.100 dtn | 1 | Liquid |
| HCP (*E. coli*) Biotin-labelled Antibody | Blue | A40034.100 dtn | 1 | Liquid |
| HCP (*E. coli*) Standard | Blue with red septum | A06034.1 ea | 2 | Liquid |
| HRP Substrate Solution (TMB) | Black | A09034.100 dtn | 1 | Liquid |
| Stop Solution | Yellow | A22000.13 mL | 1 | Liquid |
| HRP ELISA Buffer | Blue | A07034.1 ea | 1 | Lyophilised |
| Wash Buffer concentrated 400x | Silver | A17000.1 ea | 1 | Liquid |
| Tween 20 | Transparent | A12000.1 ea | 1 | Liquid |
| Technical Booklet | - | A11034 | 1 | - |
| Well cover Sheet | - | - | 1 | - |

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 40 samples in duplicate.
If you want to use the kit in two times, one additional vial of Standard is provided.

# Precaution for use

**Users are recommended to carefully read all instructions for use before starting work.**

Each time a new pipette tip is used, aspirate a sample or reagent and expel it back into the same vessel. Repeat this operation two or three times before distribution in order to equilibrate the pipette tip.

- For research laboratory use only
- Not for human diagnostic use
- Do not pipet liquids by mouth
- Do not use kit components beyond the expiration date
- Do not eat, drink or smoke in area where kit reagents are handled
- Avoid splashing

Stop Solution and Substrate Solution are harmful solutions. To avoid any contact, wear eye, hand, face and clothing protection when handling these.

Wearing lab gloves, laboratory coat and eye protection glasses is recommended when assaying kit materials and samples.

## Temperature

Unless otherwise specified, all the experiments are done at room temperature (RT), which is around +20°C. Working at +25°C or more affects the assay and decreases its efficiency.

# Background

## Host Cell Proteins [1, 2, 3].

Impurity assessment is a key step during the drug development production of recombinant proteins, including therapeutic proteins and biopharmaceuticals.

Specific impurities coming from the cells mediating the protein expression, known as "Host Cell Proteins (HCP)", are generated and need to be removed. *E. coli* is commonly used as a production system because it is relatively simple and cost-effective.

This kit is intended for use in assessing relative quantities of *E. coli* HCP in manufactured or research bioproducts.

Polyclonal antibodies used in this kit have been generated against several strains of *E. coli* and specifically selected for their recognition of a large spectrum of *E. coli* proteins. Thus, this kit can be considered as generic and allows a relative-quantitative determination of *E. coli* HCP in many types of samples, such as samples issued from the purification process (HCP clearance), process control, quality control or product release.

Using this kit, HCP concentration is measured in ng/mL (HCP equivalent is extrapolated from a standard curve). Conventionally, the HCP content in a product will finally be expressed in ng/mg, where ng represents HCP mass and mg represents the product mass.

Note that, contrary to the concentration measurement of the product, the HCP signal is only reflective of antibody binding and does not strictly reflect the mass of HCP.

This kit has been successfully validated for recovery and precision using reconstituted HCP samples and tested against different final products. Given the diversity of final products, all potential matrix effects cannot be known and it is recommended that you test the suitability of the kit with your own HCP samples in your laboratory.
This kit should be used as one part of your complete HCP analysis.

### > *Limitation*

Generic kits are limited by both their validated relative sensitivity and their specificity against HCP species possibly present in the products to be characterized.

The specific development and validation of methods for HCP characterization appropriate to your production and purification process is recommended (especially in the later phases of product development). In such cases, feel free to get back to us.

# Principle of the assay

This Enzyme Immunometric Assay (ELISA) is based on the sandwich technique.

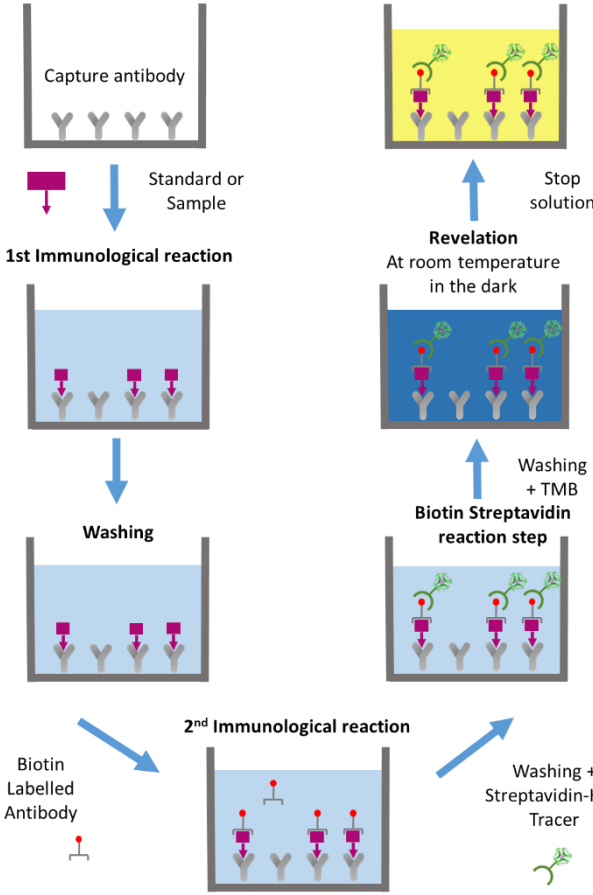
The plate supplied is coated with polyclonal antibodies (capture antibody) specific to HCP (*E. coli*).

HCP (*E. coli*) from the standards or the samples is going to bind to the polyclonal antibodies coated on the plate and then is detected by a second polyclonal antibody labelled with biotin also specific to HCP (*E. coli*). The immunological complex is revealed by the interaction between biotin and streptavidin labelled with HRP (Tracer).

The HCP (*E. coli*) content is then determined by measuring the enzymatic activity of the HRP using the TMB solution. The tracer acts on TMB reagent to form a yellow compound after the reaction has been stopped.

The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of HCP (*E. coli*) present in the well during the immunological incubation.

The principle of the essay is summarised below:



# Assay validation and characteristics

The Enzyme Immunometric assay of *E. coli* HCP has been validated for its use in HRP ELISA Buffer.

For additional information regarding the validation of immunoassay for protein biomarkers in biological samples, please refer to bibliography ***[4, 5]***.

- The **Limit of Detection (LOD)**, calculated as the concentration of HCP (*E. coli*) corresponding to the NSB absorbance average plus three standard deviations was estimated at 2 ng/mL.

- The **Limit of Quantification** was assessed by the concentration corresponding to absorbance signal plus 10 standard deviations. It was estimated at 4 ng/mL.

- **Precision and recovery** in HRP ELISA Buffer are based on 3 series with 2 determinations

| HCP Standard (ng/mL) | % C.V. intra-assay | % C.V. inter-assay | % Recovery |
|---|---|---|---|
| 111 | 19.5 | 19.5 | 97.9 |
| 333 | 19.3 | 20.1 | 129.6 |
| 1000 | 7.7 | 10.1 | 116 |
| Internal Control | 13.0 | 26.8 | N/A |

- **Specificity**

Since the cellular protein content of many different species can present a high percent of homology, cross-reactivity is expected not only with other strains of *E. coli* but with many cellular systems.

## Materials and equipment required

In addition to standard laboratory equipment, the following materials are required:

For the assay:

- Precision micropipettes (20 to 1000 µL)
- Spectrophotometer plate reader (450 and 690 nm filter)
- Microplate washer (or wash bottles)
- Orbital microplate shaker
- Multichannel pipette and disposable tips 30-300µL
- UltraPure water (item number #A07001.1L)
- Polypropylene tubes

Water used to prepare all ELISA reagents and buffers must be UltraPure (deionized & free from organic contaminant traces).
Do not use distilled water, HPLC-grade water or sterile water.

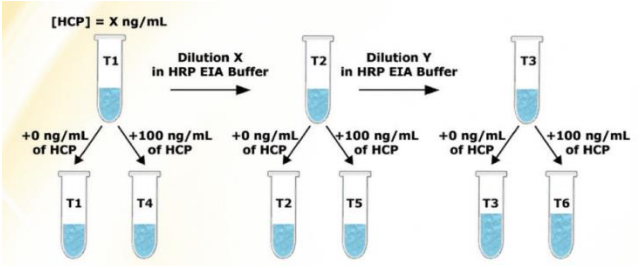
- UltraPure water may be purchased from Bertin Bioreagent (item #A07001.1L).

# Sample collection and preparation

**Make sure all buffers used for diluting samples are sodium azide** ($NaN_3$) free since it is an inhibitor of the HRP activity.

Regarding the diversity of final product, matrix effect cannot be guaranteed and it is recommended to test the suitability of the kit for use with your samples. It is advised to test several dilutions of the sample.

To do so, split a sample in two and spike one with a known amount (for instance 100 ng/mL or less) of standard (*E. coli* lysate as equivalent of HCP reference). Performing this on different dilutions of your samples will allow you to define the minimal dilution for your sample.




Assay HCP concentration for tubes 1 to 6.

No matrix effect could be considered at the dilution where the spiking will be recovered at about 25-30%.

$$\frac{\text{(measured concentration - theoretical concentration)}}{\text{theoretical concentration}} \times 100$$

Once the minimal dilution is determined, at least 3 dilution points will be ideally selected to estimate the HCP content (dilution linearity assessment).

When the sample matrix is not available, HCP content in the samples can be assessed by the standard addition method spiking various known quantity of HCP standard in the unknown sample.

The optimal working range with the lowest interferences will then be assessed in the linear range, and HCP content will be extrapolated at Y-intercept.

## Reagent preparation

Each kit contains sufficient reagents for 96 wells. This allows for the construction of one standard curve in duplicate and the assay of 40 samples in duplicate.

All reagents need to be brought to room temperature, around +20°C, prior to the assay.

### HRP ELISA Buffer

Reconstitute the vial #A07034 with 50 mL of UltraPure water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

*Stability at 4°C: +4 months*

## HCP (*E. coli*) Standard

The vial #A06034 is provided as ready to use.

The concentration of the first standard (S1) is 3000 ng/mL. Prepare six propylene tubes (for the six other standards) and add 500 µL of HRP ELISA Buffer into each tube. Then prepare the standards by serial dilution as follows:

| Standard | Volume of Standard | Volume of HRP ELISA Buffer | Standard concentration ng/mL |
|---|---|---|---|
| S1 | - | - | 3000.0 |
| S2 | 250 µL of S1 | 500 µL | 1000.0 |
| S3 | 250 µL of S2 | 500 µL | 333.3 |
| S4 | 250 µL of S3 | 500 µL | 111.1 |
| S5 | 250 µL of S4 | 500 µL | 37.0 |
| S6 | 250 µL of S5 | 500 µL | 12.3 |
| S7 | 250 µL of S6 | 500 µL | 4.1 |

*Do not store the diluted standards*

## Wash Buffer

Dilute 1 mL of Concentrated Wash Buffer #A17000 with 400 mL of UltraPure water.
Add 200 µL of Tween20 #A12000.
Use a magnetic stirring bar to mix the content.
*Stability at +4°C: 1 month*

# Assay procedure

It is recommended to perform the assay in duplicate and to follow the instructions hereafter.

## Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.

Open the plate pouch and select sufficient strips for your assay and place the unused strips back in the pouch, store at +4°C for 1 month maximum.

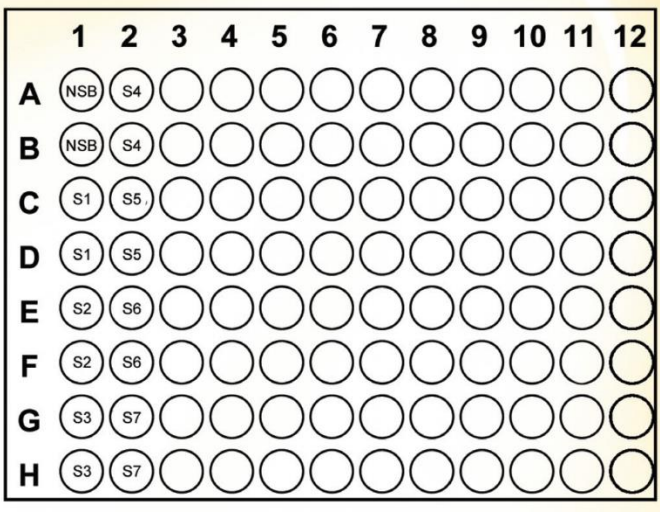
Rinse each well 5 times with Wash Buffer (300 µL/well).

Just before distributing the reagents and samples, remove the buffer from the wells by inverting the plate and shaking out the last drops on a paper towel.

## Plate set-up

A plate set-up is suggested hereafter.

The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.




NSB: Non Specific Binding S1-S7: Standards 1-7

### Pipetting the reagents

All samples and reagents must reach room temperature prior performing the assay.

Use different tips to pipet the buffer, standard, sample, tracer, biotin-labelled antibody and other reagents.

Before pipetting, equilibrate the pipette tips in each reagent. Do not touch the liquid already in the well when expelling with the pipette tip.

### *HRP ELISA Buffer*

Dispense 100 µL to Non Specific Binding (NSB) wells.

### *HCP (E. coli) Standards*

Dispense 100 µL of each of the seven standards (S7 to S1) in duplicate to appropriate wells.

Start with the lowest concentration standard (S7) and equilibrate the tip in the next higher standard before pipetting.

### *Samples*

Dispense 100 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in HRP ELISA Buffer.

### *Incubating the plate*

Cover the plate with adhesive film and incubate for 2 hours at room temperature (around 20°C) under agitation at 650 rpm (with an orbital shaker).

## Washing the plate

Empty the plate by turning over. Rinse each wells 5 times with 300 µL Wash Buffer. At the end, empty the plate and blot it on a paper towel to discard any trace of liquid.

## Pipetting the reagents

### *HCP (E. coli) Biotin-labelled Antibody*

Dispense 100 µL of HCP (*E. coli*) Biotin-labelled Antibody #A40034 into each well.

### Incubating the plate

Cover the plate with adhesive film and incubate for 2 hours at room temperature (around 20°C) under agitation at 650 rpm (with an orbital shaker).

### Washing the plate

Empty the plate by turning over. Rinse each wells 5 times with 300 µL Wash Buffer. At the end, empty the plate and blot it on a paper towel to discard any trace of liquid.

### Pipetting the reagents

- **Streptavidin-HRP Tracer**

Dispense 100 µL of Streptavidin-HRP Tracer #A22010 into each well.

### Incubating the plate

Cover the plate with adhesive film and incubate for 30 minutes at room temperature (around 20°C) without agitation.

### Washing the plate

Empty the plate by turning over. Rinse each wells 5 times with 300 µL Wash Buffer. At the end, empty the plate and blot it on a paper towel to discard any trace of liquid.

### Developing and reading the plate

Dispense 100 µL of HRP Substracte Solution #A09034 into each well incubate the plate in darkness at room temperature for 30 minutes without agitation.

Stop the colour development by adding 50 µL of Stop Solution #A22000 into each well.

Read the plate at 450 nm and at 690 nm within 5 minutes.

## Assay procedure summary

| | NSB | Standard | Sample |
|---|---|---|---|
| HRP ELISA Buffer | 100 | - | - |
| Standard | - | 100 | - |
| Sample | - | - | 100 |
| Cover plate, incubate 2 hours at RT under agitation at 650 rpm | | | |
| Wash strips 5 times with 300 µL/well. Dry on absorbent paper | | | |
| HCP (*E. coli*) Biotin-labelled antibody | 100 | | |
| Cover plate, incubate 2 hours at RT under agitation at 650 rpm | | | |
| Wash strips 5 times with 300 µL/well. Dry on absorbent paper | | | |
| Streptavidin HRP Tracer | 100 | | |
| Cover plate, incubate 30 minutes at RT | | | |
| Wash strips 5 times with 300 µL/well. Dry on absorbent paper | | | |
| HRP Substrate Solution | 100 | | |
| Incubate 30 minutes in the dark at RT without agitation | | | |
| Stop Solution | 50 | | |
| Read the plate at 450 and 690 nm | | | |

# Data analysis

Make sure that your plate reader has subtracted the absorbance readings at 690 nm from those at 450 nm well to well. If it is not the case, please do it.

- Most plate readers are supplied with a curve-fitting software capable of plotting this type of data. If you have this type of software, we recommend using it. Refer to it for further information and plot the absorbance for each standard (Y axis) versus the concentration (X axis) using a 4 -parameter logistic fitting.
- If you have not this type of software, calculate the average absorbance for each NSB, standard and sample.
- For each standard, plot the absorbance on Y axis versus the concentration on X axis (using a semi-log graph). Draw a best-fit line through the points. To determine the concentration of your samples, find the absorbance value of each sample on the Y axis.
- Read the corresponding value on the X axis which is the concentration of your unknown sample.
- Do not forget to integrate the dilution factor of your samples
- It is recommended to estimate the HCP content in your sample using at least 2 to 3 dilution points (in duplicate) selected in the linear range of the curve.
- Samples with a concentration greater than 3000 ng/mL should be re-assayed after dilution in HRP ELISA Buffer.

- For assessing HCP content in samples using the standard addition method, plot the calculated concentration of the spiked samples (Y axis) versus the added volume (X axis) and extrapolate the linear range of the curve to read the unknown concentration at Y-intercept.

## Acceptable range

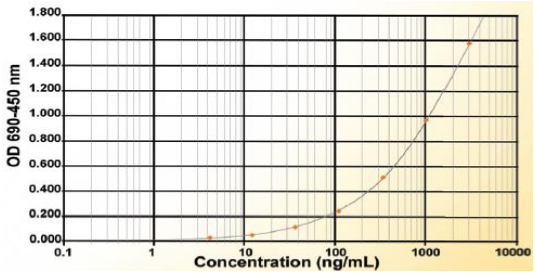
- NSB absorbance < 100 mAU
- Maximum Absorbance < 3000 mAU

## Typical results

The following data are for demonstration purpose only. Your data may be different and still correct.
The data were obtained using all reagents as supplied in this kit under the following conditions: 15 minutes developing at RT, reading at 450/690 nm. A 4PL fitting was used to determine the concentrations.

| HCP Standard (ng/mL) | Absorbance (mAU) |
|---|---|
| 3000 | 1581 |
| 1000 | 968 |
| 333 | 515 |
| 111 | 242 |
| 37 | 115 |
| 12.3 | 52 |
| 4.1 | 31 |
| 0 (NSB) | 16 |

Typical HCP standard curve




# Troubleshooting

### > *Absorbance values are too low:*

- Organic contamination of water,
- One reagent has not been dispensed,
- Incorrect preparation / dilution,
- Assay performed before reagents reached room temperature,
- Reading time not long enough.
- Check that no sodium azide is into the samples.

### > *High signal and background in all wells:*

- Inefficient washing,
- Overdeveloping (incubation time should be reduced),
- High ambient temperature.

> ***High dispersion of duplicates:***

- Poor pipetting,
- Irregular plate washing.

These are a few examples of troubles that may occur.

If further information or explanation is needed, please contact Bertin Bioreagent Technical Support by phone on +33 (0)139 306 036 or by E-mail tech@bertin-bioreagent.com. Please have batch number of the kit (see outside the box) ready to provide to the technical support.

Bertin Bioreagent offers ELISA Training kit #B05005. Feel free to contact our Technical Support. We are always happy to hearing from you.

## Bibliography

**1.** US Pharmacopeial Convention
Residual Host Cell Protein Measurement in Biopharmaceuticals.
*HCP IMMUNOASSAY METHOD VALIDATION/4.3 Sample Linearity/Table 4 - Second Supplement to USP38–NF33 – p. 7647 – 1st April 2016*

**2.** Tscheliessnig AL. *et al*.
Host cell protein analysis in therapeutic protein bioprocessing - methods and applications.
*Biotechnol J. 2013, 8(6):655-70.*

**3.** Wang X *et al*.
Host cell proteins in biologics development: Identification, quantitation and risk assessment.

*Biotechnol Bioeng. 2009, 103(3):446-58.*

**4.** Valentin MA., Ma S., Zhao A. *et al.*
Validation of immunoassay for protein biomarkers: Bioanalytical study plan implementation to support pre-clinical and clinical studies.
*J Pharm Biomed Anal. (2011) 55(5) : 869-877*

**5.** European Medicines Agency
*Guideline on bioanalytical method validation, 21 July 2011*

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With 30 years of experience, Bertin Bioreagent develops and sells best-in-class kits and products for life science research labs. Our scientist team innovate each day to tailor biomarker assays, pre-analytical products, kits, antibodies and biochemicals that are ready to use, fully validated with a strict quality control.
We strive to address a broad range of research interest: inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, pain, prion diseases.

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