HOST CELL PROTEIN - HCP (E. *coli*)

A brand name of

bertin pharma

For laboratory research use only. Not for human or veterinary diagnostic use.

Bertin Pharma also markets pre-analytical products, EIA kits, antibodies, CYP450s & biochemicals for:

- Cardiology / Hypertension
- Diabetes / Obesity
- Endocrinology / Metabolism
- Inflammation
- Pharmacology
- Psychopharmacology
- Nitric Oxide
- Oncology / Apoptosis
- Oxidative injury
- Cell signaling
- Drug metabolism

Do not hesitate to contact our after-sales services for further information at bioreagent@bertinpharma.com

European patent # 89 139 552
U.S. patent # 50 47 330

Host cell protein - HCP (E.*coli*)
Enzyme Immunoassay kit
#A05034.96 wells

For research laboratory use only
Not for human diagnostic use

This assay has been developed & validated
by Bertin Pharma

Fabriqué en France
Made in France

#A11034
Version: 0116

Table of contents

96 wells
Storage: +2 - +8°C
Expiry date: stated on the package

This kit contains:

Designation	Colour of cap	Item #	Quantity per kit	Form
A covered 96 well Microtiter plate, pre-coated with HCP (E.*coli*)	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 EIA Buffer	blue	A0703.1 ea	1	Lyophilized
Concentrated Wash Buffer 400x	silver	A17000.1 ea	1	Liquid
Tween 20	transparent	A12000.1 ea	1	Liquid
HRP Substrate Solution (TMB)	black	A09000_50.100 dtn	1	Liquid
Stop Solution	yellow	A22000.13 mL	1	Liquid
Instruction booklet	-	A11034	1	-
Well cover sheet	-	-	-	-

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.

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 in which 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 gloves, laboratory coat and glasses is recommended when assaying kit materials and samples.

Temperature

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

Background

Host Cell Proteins

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

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 for use 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 latter phases of product development). In such cases, feel free to get back to us.

Principle of the assay

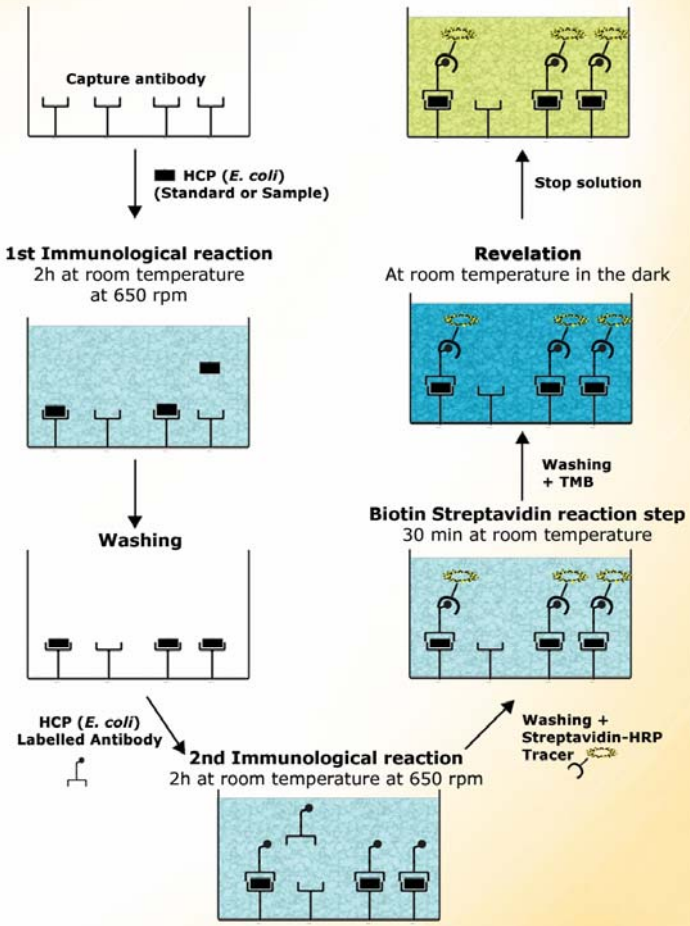
This Enzyme Immunometric Assay (EIA) is based on the sandwich technique.
The plate supplied is coated with polyclonal antibodies (capture antibody) specific to HCP (E.*coli*).

HCP (E.*coli*) from the standards or the samples is going to bind to the polyclonal antibody coated on the plate and then is detected by a second polyclonal antibody labelled with biotin also specific for 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 assay is summarised below:



Materials and equipment required

In addition to standard laboratory equipment, the following material is required:

For the assay:

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

Water used to prepare all EIA reagents and buffers must be Ultra Pure, deionized & free from organic contaminants traces.
Do not use distilled water, HPLC-grade water or sterile water.

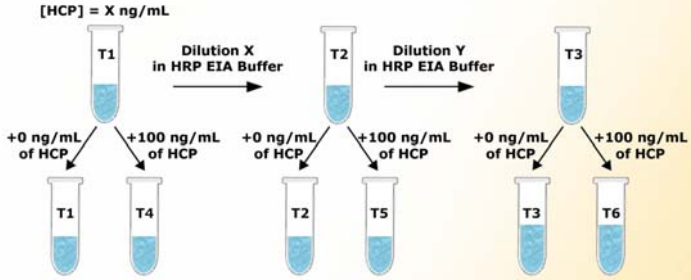
- UltraPure water may be purchased from Bertin Pharma (item #A07001.1L)

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 samples 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 .



Measure 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

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

HRP EIA 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 month

HCP (E.*coli*) Standard

The vial #A06034 is sold ready to use
The concentration of the first standard (S1) is 3 000 ng/mL. Prepare six propylene tubes (for the six other standards) and add 500 µL of HRP EIA Buffer into each tube. Then prepare the standards by serial dilution as follows:

Standard	Volume of Standard	Volume of HRP EIA Buffer	Standard concentration ng/mL
S1	-	-	3 000.0 ng/mL
S2	250 µL of S1	500 µL	1 000.0 ng/mL
S3	250 µL of S2	500 µL	333.3 ng/mL
S4	250 µL of S3	500 µL	111.1 ng/mL
S5	250 µL of S4	500 µL	37.0 ng/mL
S6	250 µL of S5	500 µL	12.3 ng/mL
S7	250 µL of S6	500 µL	4.1 ng/mL

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 assays in duplicate and to follow the instructions hereafter.

Plate preparation

Prepare the Wash Buffer as indicated in the reagent preparation section.
Open the plate packet and select the sufficient strips for your assay and place the unused strips back in the packet, store at +4°C for 1 month maximum.

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

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

Distribution of reagents and samples

A plate set-up is suggested on the following page.
The contents of each well may be recorded on the template sheet provided at the end of this technical booklet.

Pipetting the reagents

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

Use different tips to pipette 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 expeling with the pipette tip.

> HRP EIA Buffer

Dispense 100 µL to Non Specific Binding (NSB).

> HCP (E.*coli*) Standard

Dispense 100 µL of each of the seven standards S1 to S7 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 EIA Buffer.

▷ Incubating the plate

Cover the plate with adhesive film and incubate for 2 hours at room temperature (around 25°C) under agitation at 650 rpm (with an orbital shaker).

Rinse each 5 times with 300 µL/well. Dry on absorbent paper.

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

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

Incubating the plate

Cover the plate with adhesive film and incubate for 2 hours at room temperature (around 25°C) under agitation at 650 rpm (with an orbital shaker).

Rinse each well 5 times with 300 µL/well. Dry on absorbent paper.

Streptavidin-HRP Tracer

Dispense 100 µL to each well.

Incubating the plate

Cover the plate with adhesive film and incubate for 30 minutes at room temperature (around 25°C) without agitation.

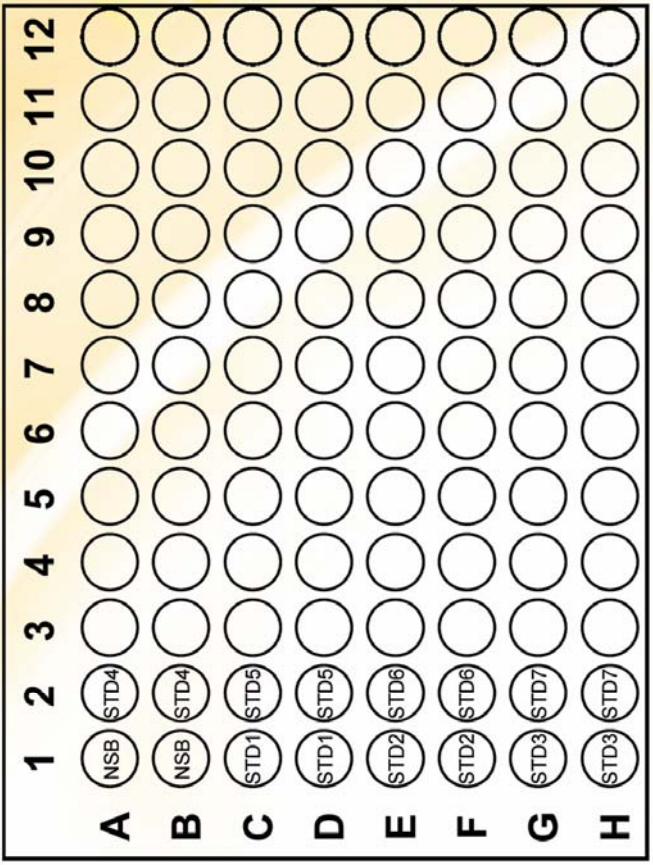
Rinse each well 5 times with 300 µL/well. Dry on absorbent paper.

Developing and reading the plate

Dispense 100 µL of TMB substrate into each well and incubate the plate in darkness at room temperature for 30 minutes without agitation.

Stop the colour development by adding 50µL of Stop Solution in each well.

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



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

*: Samples

Enzyme Immunoassay Protocole (volumes are in µL)			
	NSB	Standard	Sample
HRP EIA 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 curve-fitting software capable of graphing these 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 3 000 ng/mL should be re-assayed after dilution in EIA HRP Buffer.

- Samples with a concentration greater than 3 000 ng/mL should be re-assayed after dilution in EIA HRP Buffer.
- For assessing HCP content in the 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.

Typical results

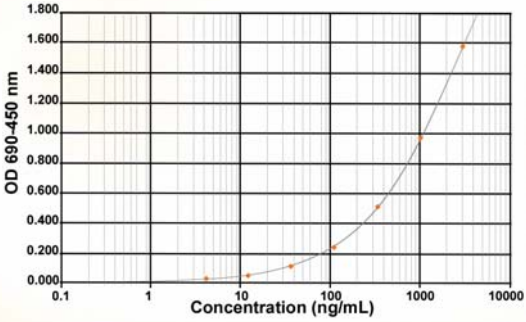
The following data are for demonstration purpose only. Your data may be different and still correct.

- Non Specific Binding <100 mAU
- Maximum Absorbance < 3 000 mAU

These 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



Assay validation and characteristics

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

> The limit of detection, calculated as the concentration of HCP (E.*coli*) corresponding to the NSB absorbance average plus three standard. It was estimated at about 2 ng/mL.

> The limit of quantification was assessed by the concentration corresponding to absorbance signal +10 standard deviation. It was estimated at about 4 ng/mL.

> Precision and recovery in HRP EIA Buffer are based on 3 series with 2 determinations

HCP standard (ng/mL)	% C.V. intra-assay	% CV 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.

Assay troubleshooting

- **Absorbance values are too low**: organic contamination of water, or one reagent has not been dispensed, or incorrect preparation, or assay performed before reagents reached room temperature, or reading time not long enough.

- **High signal and background in all wells**: Inefficient washing or overdeveloping (incubation time should be reduced) or high ambiant temperature.

- **High dispersion of duplicates**: Poor pipetting technique or irregular plate washing.

These are a few examples of trouble shooting that may occur. If you need further explanation, Bertin Pharma will be happy to assist you. Feel free to contact our technical support staff by phone (+33 (0)139 306 036), fax (+33 (0)139 306 299) or E-mail (bioreagent@bertinpharma.com), and be sure to indicate the batch number of the kit (see outside the box).

Bertin Pharma proposes EIA Training kit #B05005 and EIA workshop upon request. For further information, please contact our Marketing Department by phone (+33 (0)139 306 260) or E-mail (marketing@bertinpharma.com).

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.
   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.

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

Bertin Pharma, over the last decades, has been developing and marketing over 100 biomarker assays, pre-analytical products, kits, antibodies and bio-chemicals thanks to its innovative work in research and development.
Our core areas are orientated to inflammation, oxidative injury, endocrinology, diabetes, obesity, hypertension, neurodegenerative diseases, HIV, prion diseases, pharmacokinetics and metabolism.

Bertin Pharma is active worldwide either with direct sales or through our qualified and trained international distribution network from the United States to Japan.

We are able to provide you with local technical support to use at ease our products.

For further information, please send your request to
bioreagent@bertinpharma.com

bertin pharma

care. innovate. deliver.

Parc d'activités du Pas du Lac - 10 bis avenue Ampère
F-78180 Montigny-le-Bretonneux - France
Tel: +33 (0)139 306 036 - Fax: +33 (0)139 306 299
bioreagent@bertinpharma.com - www.bertinpharma.com