HEK293|360-HCP ELISA

ELISA for the Determination of HEK293 HCP

Instruction Leaflet

THIS PRODUCT IS INTENDED FOR LABORATORY AND RESEARCH USE ONLY. IT IS NOT SUITABLE FOR DIAGNOSTIC OR THERAPEUTIC APPLICATIONS.

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1 Intended Use

The HEK293|360-HCP ELISA (HEK293 host-cell-protein-specific enzyme-linked immunosorbent assay) is provided as a ready-to-use ELISA kit to be performed in 96 well microtest plates. This generic assay is intended for the quantitative in-vitro measurement of process-related HCP impurities derived from recombinant production processes based on HEK293 (human embryonic kidney) cell line. Two kit types are available which were developed by using either the supernatant (SN) or the cell lysate (CL) derived from HEK293 mock fermentation as antigen fractions for antibody generation.

2 Test Principle

This quantitative impurity test method (HEK293|360-HCP ELISA) is based on a sandwich enzyme immunoassay to be performed in 96 well microtest plates. The polyclonal antibodies used were raised by immunization (rabbit) and isolated by affinity purification using the respective HEK293 HCP mock material as the antigen (cf. section 1). Samples, which may potentially contain the corresponding HEK293 HCP impurities (analyte), are incubated in microtest plate wells pre-coated with the affinity-purified HEK293 HCP-specific capture antibody alongside a standard curve of different analyte concentrations (1.563 – 100 ng/mL). After incubation and a plate washing step in which unbound components are removed, the biotin-conjugated detector antibody (the same which is used as capture antibody) is added. After further washing steps, the bound detector antibody in turn reacts with the enzyme conjugate (streptavidin-conjugated peroxidase), which acts as the tracer. After a final washing step, the reaction is developed with tetramethylbenzidine (TMB) resulting in a blue color. Eventually, the reaction is stopped by addition of sulfuric acid converting the blue color

to yellow. The optical density is then measured photometrically at a wavelength of 450 nm using reference wavelengths between 620 – 690 nm (recommended 630 nm).

The optical density is proportional to the analyte concentration in the wells. The analyte concentration in samples can be calculated based on the corresponding standard curve of known concentrations.

3 Materials and Equipment

3.1 ELISA Kit Reagents

This ELISA kit contains all reagents required to perform the HEK293|360-HCP ELISA as listed below. The ELISA kit reagents are to be stored at 2 – 8°C or below -60°C (Kit Standard) and used before their lot-specific expiration date.

Reagents	Details	Quantity
Microtest Plate (pre-coated)	pre-coated with affinity-purified HEK293 HCP-specific capture antibody (polyclonal, rabbit), dried and sealed in foil bag with desiccant, ready-to-use	96 wells
Washing Buffer (10x)	Tris-based washing buffer for immunoassays, (“BlueCap Solutions”, BioGenes GmbH, Cat. No. S210), working dilution 1:10	100 mL
Assay Buffer (10x)	Tris-based washing buffer for immunoassays, (“BlueCap Solutions”, BioGenes GmbH, Cat. No. S210), working dilution 1:10	20 mL
Kit Standard (10 µg/mL)	HEK293 HCP mock material in stabilized solution (protein concentration determined using the Bradford assay, not BCA), **store below -60°C immediately upon receipt**	0.1 mL
Detector Antibody (100x)	affinity-purified, biotin-conjugated HEK293 HCP-specific detector antibody (polyclonal, rabbit) in stabilized solution, working dilution 1:100	0.3 mL
Enzyme Conjugate (100x)	streptavidin-conjugated peroxidase in stabilized solution, working dilution 1:100	0.3 mL
Substrate Solution	TMB ONE substrate solution, ready-to-use	15 mL
Stop Solution	0.5 M sulfuric acid, ready-to-use	15 mL

3.2 Reagents, Materials and Equipment Not Provided in the ELISA Kit

- microplate washer (8-channel or 96-channel)
- orbital microplate shaker (400 – 600 rpm) and vortex mixer
- precision pipettes (adjustable volumes, e.g. 10 – 10,000 µL)
- multichannel pipette (8-channel, 100 µL or adjustable volume, e.g. 30 – 300 µL)
- multichannel microplate reader for optical density measurement at 450 nm (reference wavelength adjustable between 620 – 690 nm, recommended 630 nm)
- pipette tips (volumes of e.g. 10 – 10,000 µL)
- suitable reaction tubes (e.g. 1.7 mL, 2 mL, 5 mL, 15 mL, 50 mL)
- suitable reagent bottles and beakers
- suitable reagent reservoirs for effective multichannel pipetting
- suitable lids/adhesive foil for covering microtest plates
- absorbent paper towels for removing residual liquid after microtest plate washing
- ultrapure water for dilution of the 10x concentrate of the washing/assay buffer

4 Warnings and Precautions

- This ELISA kit is intended for in-vitro laboratory and research use only and should solely be used by qualified personnel.
- Before performing 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.
- Contamination of solutions and microtest plate wells with proteins of human origin should be avoided during assay execution
- Some reagents of the ELISA kit contain a mixture of CMIT/MIT (Methylchloroisothiazolinone/Methylisothiazolinone) as preservative. These reagents may cause 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 M 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 reagents and solutions have to be treated as potentially hazardous waste according to national safety guidelines and regulations.

5 Procedures

5.1 Reagent Preparation

In general, the 1x washing/assay buffer, the 1x detector antibody and enzyme conjugate working solutions and the different standards and samples should be prepared immediately before use. Each of these solutions should only be used on the day of preparation.

5.1.1 Preparation of the 1x Washing Buffer

Dilute the 10x concentrate of the washing buffer with ultrapure water before use (e.g. 100 mL of 10x concentrate with 900 mL ultrapure water).

5.1.2 Preparation of the 1x Assay Buffer

Dilute the 10x concentrate of the assay buffer with ultrapure water before use (e.g. 20 mL of 10x concentrate with 180 mL ultrapure water).

5.1.3 Preparation of the Standards

The kit standard is thawed at 18 – 26°C immediately before use and diluted in 1x assay buffer producing the different standards according to the following scheme. Pure 1x assay buffer is used as assay blank. The volumes can be adjusted depending on the number of microtest plates to be analyzed.

Solution ID	Nominal Concentration [ng/mL]	Volume [µL]	of Solution ID	Volume 1x Assay Buffer [µL]
Kit Standard	**10,000**	-	-	-
S8	100	10	**Kit Standard**	990
S7	50	500	S8	500
S6	25	500	S7	500
S5	12.5	500	S6	500
S4	6.25	500	S5	500
S3	3.125	500	S4	500
S2	1.563	500	S3	500
S1 (assay blank)	0	0	-	500

S: standard

5.1.4 Preparation of Samples

Dilute the samples with 1x assay buffer before ELISA measurement. The minimum required dilutions (MRD) have to be determined by the user for each sample type depending on the analyte content and by taking the assay working range into account. Furthermore, an accurate and precise analyte quantification has to be demonstrated for the selected dilution range.

5.1.5 Preparation of the 1x Detector Antibody Working Solution

Dilute the 100x concentrate of the detector antibody with 1x assay buffer before use (e.g. 120 µL of 100x concentrate with 12 mL 1x assay buffer for one microtest plate).

5.1.6 Preparation of the 1x Enzyme Conjugate Working Solution

Dilute the 100x concentrate of the enzyme conjugate with 1x assay buffer before use (e.g. 120 µL of 100x concentrate with 12 mL 1x assay buffer for one microtest plate).

5.2 General ELISA Procedure

All steps of the ELISA are performed at temperatures between 18 – 26°C. Allow all materials and reagents to reach this temperature before opening and using them. During all incubation steps, the plates should be covered with a lid to prevent evaporation and contamination of solutions. All plate washing steps should be performed with an automatic microplate washer and the respective washing programs. Plate shaking is to be performed at 400 – 600 rpm using a suitable microplate shaker.

Step	Reagent	Volume per Well	Incubation Time
Antigen Incubation	standards, assay controls and samples (duplicate or triplicate wells are recommended)	100 µL	2 h (continuous shaking)
Washing Step	1x washing buffer	4x 250 µL	
Detector Antibody Incubation	1x detector antibody working solution	100 µL	1 h (continuous shaking)
Washing Step	1x washing buffer	4x 250 µL	
Enzyme Conjugate Incubation	1x enzyme conjugate working solution	100 µL	20 min (continuous shaking)
Washing Step	1x washing buffer	4x 250 µL	
Substrate Incubation	substrate solution (ready-to-use)	100 µL	10 – 15 min (continuous shaking)[1]
Stop Reaction	stop solution (ready-to-use)	100 µL	readout OD_{450}[2] (vs. OD_{630}) within 15 min after stopping

[1]: If color development is too low after 15 min the substrate incubation can be extended up to 30 min.

[2]: The optical density at 450 nm (OD_{450}) is measured with a reference wavelength between 620 – 690 nm (recommended 630 nm). For further data evaluation (e.g. standard curve regression, (back)calculation of the analyte concentration), the value obtained at the reference wavelength for each well is subtracted from the corresponding value measured at 450 nm.

5.3 Measurement and Calculations

The optical density at a wavelength of 450 nm is to be measured with a reference wavelength between 620 – 690 nm (recommended 630 nm) using a suitable multichannel microplate reader and the corresponding software. The standard curves should be generated employing a nonlinear regression mode (four-parameter equation). The analyte concentrations are (back)calculated by the microplate reader software for the different standards and samples.

6 Troubleshooting

Possible reasons (explanations) for lacking ELISA performance are listed below.

Low reactivity throughout the whole plate

- omission of incubation steps and/ or reagents
- 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

High reactivity and assay background throughout the whole plate

- improper washing steps
- inadequate storage or preparation of ELISA components/ reagents
- overdevelopment of the plate with substrate solution before stopping
- contamination of the substrate solution

Poor intra-assay precision (high CV of replicate wells)

- improper washing steps
- insufficient mixing of solutions
- inhomogeneous samples containing aggregates/ precipitates
- contamination of solutions and microtest plate wells with human proteins

7 Contact

Were you satisfied with the results obtained from the use of our HEK293|360-HCP ELISA? Would you like to try further kit types or order specific buffers? Do you need help with any of the steps above or further information? Our specialized team will be happy to assist you!

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