BioGenes ASSAYS

Short Report

Qualification Study

CHO|360-HCP ELISA (Type A to D)

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1 Introduction

The purpose was the development of an enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of process-related CHO-HCP.
This ELISA (referred to as the CHO|360-HCP ELISA) was successfully developed as a kit of four different types A to D, based on four different polyclonal affinity purified antibody preparations as capture and detector antibody.
The method based on the sandwich ELISA principle with several sequential incubations steps in an antibody coated 96 well microtest plate.
After the first incubation step for HCP binding by the capture antibody and several washings, bound HCP is detected with the biotinylated detector antibody (the same antibody as the capture).This biotin-conjugated antibody is then in turn detected after several washing steps with a Streptavidin-Peroxidase conjugate. After further washings, the plate is incubated with the substrate Tetramethylbenzidine. The enzymatic color reaction is stopped by adding Sulphuric acid and the color intensity, which is proportional to the HCP concentration, is measured photometrically.
The CHO|360-HCP ELISA method was qualified at BioGenes according to the respective ICH-Guideline ICH Q2(R1) ("Validation of analytical procedures: text and methodology") with slightly deviations due to the nature of the method as a bioassay.

1.1 Type of analytical procedure

The method is intended to be used for the following type of analytical procedure:

- Test for impurities – Quantitation

1.2 Validation characteristics

The following validation characteristics according to ICH Q2(R1) were tested:

- Specificity
- Accuracy
- Repeatability (Precision)
- Intermediate Precision
- Linearity
- Limit of Detection
- Limit of Quantitation
- Range

2 Materials

2.1 Reagents

Table 3: CHO|360-HCP ELISA Kit with components [No. (1) to (8)] and further reagents [(9) pp.]

No.	Reagent	Details
(1)	Microtest Plate (ready-to-use)	96 well, pre-coated with affinity-purified anti-CHO-HCP-IgG (polyclonal, rabbit or goat), dried and sealed in foil bag with desiccant ready-to-use microtest plate
(2)	Washing Buffer (10x)	TBS, pH 7.5 with Triton X-100; containing Phenol Red and ProClin®300 as preservative, 10x concentrate
(3)	Assay Buffer (ready-to-use)	TBS, pH 7.5 with Triton X-100; 1% BSA and ProClin®300 as preservative, ready-to-use solution
(4)	CHO-HCP Standard (10 µg/mL)	Stock solution of CHO-HCP in TBS with ProClin®300 as preservative,
(5)	Detector Antibody (100x)	affinity purified, biotin-conjugated anti-CHO-HCP-IgG-Biotin (polyclonal, rabbit or goat) in stabilized solution 100x concentrate
(6)	Enzyme Conjugate (100x)	Streptavidin-conjugated Peroxidase in stabilized solution, 100x concentrate
(7)	Substrate Solution (ready-to-use)	TMB One substrate solution ready-to-use solution
(8)	Stop Solution (ready-to-use)	0.5 M Sulfuric acid ready-to-use solution
(9)	CHO-HCP Standard stock solution (0.5 mg/mL)	Starting material for preparing the CHO-HCP standard of the kit, 0,5 mg/ml CHO-HCP in PBS with ProClin®300 as preservative; serves as spiking material for sample spike

2.2 Samples

For the ELISA qualification four real samples from different DSP stages and types were used.

Table 4: Sample Overview

No.	Sample Coding	Sample Type
(10)	Sample 1	IPC
(11)	Sample 2	IPC
(12)	Sample 3	IPC
(13)	Sample 4	FB

2.3 Evaluation system

The optical density was measured with the multichannel microplate reader OpsysMR™ and the corresponding software Revelation QuickLink 4.04 giving the raw data of the assays. For calculations and graphic presentation of the raw data the software GraphPad Prism® 5 was used. The raw data were imported into the respective data files of the software and the HCP calibration curves were generated by using a nonlinear regression mode, the “agonist vs. response with variable slope” (based on the four-parameter-equation). For calculating HCP concentrations and HCP recovery rates as well as different statistic parameters such as means, standard deviations (SD) and coefficients of variation (CV) the software Microsoft® Office Excel® 2007 was used.

3 Summarized Results

The results of the qualification study are summarised in the following table.

Table: Summary of the results of the qualification study for the CHO|360-HCP ELISA Kits A - D

Validation Parameter	Experiment	Result
Specificity	1-D and 2-D analysis (electrophoresis and Western blotting) Acceptance criterion: *Satisfying antigen coverage:* *antigen coverage ≥ 80%*	All 4 antibody preparations (A to D) demonstrate a satisfying coverage of the CHO|360-HCP antigen distribution in 1-D and 2-D analysis
Accuracy	Spiking approaches of three different real matrix samples with two different amounts of CHO|360-HCP Acceptance criterion: *Recovery of 100±30% of spiked antigen*	CHO|360-HCP recovery in the three spiked samples within the assay working range between 72.2% and 126.8%
Repeatability (Intra-assay Precision)	Threefold determination of three different real matrix samples with two sample dilutions in one ELISA run *Acceptance criterion:* *CV(repeatability) ≤ 15%*	Repeatability Precision of the CHO|360-HCP determination in the HCP-spiked sample within the assay working range: CV (repeatability) between 0.2% and 11.9%
Intermediate Precision (Inter-assay)	Sixfold determination (6 ELISA runs) of four different real matrix samples with two sample dilutions each *Acceptance criterion:* *CV(Intermediate precison) ≤ 20%*	Precision of the HCP determination in the HCP-spiked Sample within the assay working range: CV (Intermediate precision) between 1.1% and 15.8%
Linearity	Nonlinear regression with the 4-parameter equation *Acceptance criterion:* *- Coefficient of determination R^2 >0,9900* *Residual standard deviation S_{yx} < 0,1000*	24 standard curves were generated with the 4-parameter equation : R^2: 0.9995 – 1.0000 - S_{yx}: 0.0042 – 0.0278

Table continued		
Validation Parameter	**Experiment**	**Result**
Limit of Detection (LOD)	Part A: Definition of the Limit of Detection	The Limit of Detection of the CHO|360-HCP ELISA types A to D was defined as 0.6 ng/mL HCP.
	Part B: Confirmation of the Limit of Detection *Acceptance criterion:* *Overlapping of 3*SD ranges of OD values of LOD and blank ≤ 50%*	The Limit of Detection of the CHO|360-HCP ELISA types A to D was successfully confirmed at 0.6 ng/mL HCP by the overlapping of 3*SD ranges of OD values of LOD and blank by less than 50%
Limit of Quantitation (LOQ)	Part A: Definition of the Limit of Quantitation as the 3-fold value of LOD	The Limit of Quantitation of the CHO|360-HCP ELISA types A to D was defined as 2 ng/mL HCP.
	Part B: Confirmation of the Limit of Quantitation *Acceptance criteria :* - *HCP recovery of 100%±30%* - *CV(repeatability) ≤15%* - *No overlapping of the 3x SD range with that of the assay blank*	The Limit of Quantitation was confirmed at 2 ng/mL HCP of the CHO|360-HCP ELISA types A to D with: - HCP recovery: 81.0 - 106.5% - CV (repeatability): 5.3 – 11.4% - No overlapping of the 3*SD range with the 3*SD range of the assay blank
Working Range	Results of the LOQ confirmation approach at 2 ng/mL and 100 ng/mL as the upper limit *Acceptance criteria:* - *HCP recovery of 100%±30%* - *CV(Repeatability) ≤15*	The Working Range of the CHO|360-HCP ELISA types A to D (2 ng/mL to 100 ng/mL) was successfully confirmed with: - HCP recovery at 2 ng/mL HCP: 81.0% - 106.5 % - HCP recovery at 100 ng/mL HCP: 97.0% - 102.7% - precision at 2 ng/mL HCP: CV (repeatability): 5.3% – 11.4% - precision at 100 ng/mL HCP: CV (repeatability): 2.1% - 2.3%

4 Abbreviations

A_{450}: absorbance at 450 nm
CV: coefficient of variation
end.: endogenous
HCP: host cell protein
OD: optical density
PD pre-dilution
R^2: coefficient of determination
SD: standard deviation
S_{yx}: residual standard deviation
WD: working dilution