

Amersham™ HCPQuant™ CHO (supernatant) kit

Instructions for Use

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1 Introduction

Product code

29496737

Important user information

Read these instructions carefully before using the product.

Intended use

The product is intended for research or manufacturing use only, and must not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

All chemicals should be considered as potentially hazardous. Avoid contact with skin or eyes, in case of contact wash immediately with water. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Only trained users must use this product in accordance with the principles of good laboratory practice.

See material safety data sheets and/or safety statements for specific advice.

Storage

Store all kit components at 2°C to 8°C upon arrival. Aliquot the reconstituted protein standard and store at -20°C.

Expiry

The expiry date is shown on the package and is at least four weeks from the delivery date.

2 Background

Product description

Amersham™ HCPQuant™ CHO (supernatant) kit (29496737) is a sandwich ELISA kit designed to detect CHO-HCP contamination in process-derived samples. The kit uses purified antibodies generated against CHO-S cell supernatant derived from cells grown in chemically-defined media.

Assay description

Anti-CHO antibodies react strongly with supernatant proteins from CHO-S and CHO-K1 cell lines and can also be suitable for other CHO derivatives. Detection and quantification levels of samples derived from CHO cell lysates varies and might be limited. The impurities in process-derived samples containing CHO supernatant proteins are captured in a microtiter-type plate by using pre-coated, purified, rabbit polyclonal, CHO HCP-specific antibodies.

Detection and analysis

Captured proteins are detected using an HRP conjugated rabbit polyclonal CHO-HCP antibody. The addition of TMB results in a chromogenic change that can be measured photometrically at 450 nm with a reference wavelength at 630–650 nm. The optical density (OD) in the wells is directly proportional to the HCP concentration and correlates with the amount of HCP present in the original sample. The HCP concentration can be calculated based on the calibration curve generated with the provided CHO supernatant HCP protein standard.

3 Kit components

Component	Size
HCPQuant CHO (Sup) Antibody-coated 96- Well Strip Plate	1 plate
HCPQuant CHO (Sup) Protein Standard	1 µg/vial
HCPQuant CHO (Sup) Wash Buffer (10×)	60 mL/bottle
HCPQuant CHO (Sup) Detection Antibody	100 µL/vial
HCPQuant CHO (Sup) Sample Buffer	50 mL/bottle
HCPQuant CHO (Sup) TMB Buffer	20 mL/bottle
HCPQuant CHO (Sup) Stop Buffer	20 mL/bottle
Plate sealer	3 sheets

4 Reagents and materials required but not provided

- Microcentrifuge tubes (1.5 mL)
- Single-channel pipettes (2–20 μ L, 10–100 μ L, and 100–1000 μ L) and tips
- Multichannel pipette (50–300 μ L) and tips
- Spectrophotometer (microplate reader) capable of reading 96-well plates at 450 nm with a reference filter at 630–650 nm
- Reagent reservoirs
- Plate shaker
- Vortex mixer
- Stir plate and magnetic stir bar
- Absorbent paper
- Timer
- Polypropylene centrifuge tubes (15 mL)
- Deionized water
- Disposable gloves
- Graduated cylinder

5 Assay reagents preparation

Introduction

Prepare the wash buffer and the assay standards before beginning the assay. Prepare the detection antibody working solution just before adding it to the plate.

Prepare the wash buffer (1 ×)

Follow the steps below to prepare the wash buffer (1 ×).

Step	Action
1	Add 50 mL of HCPQuant CHO (Sup) Wash Buffer (10×) to 450 mL of deionized water.
2	Mix for at least 10 minutes using a magnetic stir bar.

The wash buffer (1 ×) can be stored at room temperature (15°C to 25°C) for up to 2 weeks, after which it should be discarded.

Prepare the assay standards

A standard curve must be generated for every assay. The stock of protein standard (HCPQuant CHO (Sup) Protein Standard) provided in the kit is enough to run several sets of assay standards at the described dilutions and the spike control tests to monitor matrix specific effects.

Follow the steps below to prepare the assay standards.

Step	Action																																				
1	Reconstitute the HCPQuant CHO (Sup) Protein Standard with 1 mL deionized water to obtain a final concentration of 1 µg/mL (stock). Tip: <i>If the protein standard is used in subsequent assays, it is recommended to aliquot the stock and store it at -20°C to avoid freeze-thaw cycles.</i>																																				
2	Follow the table below to prepare a set of standard dilutions sufficient for two replicates (100 µL per well). Table 5.1: Assay standards preparation table <table border="1"> <thead> <tr> <th>Assay standard</th> <th>Sample buffer volume (µL)</th> <th>Assay standard volume and source (µL)</th> <th>Assay standard concentration</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>400</td> <td>100 of stock</td> <td>200 ng/mL</td> </tr> <tr> <td>B</td> <td>250</td> <td>250 of A</td> <td>100 ng/mL</td> </tr> <tr> <td>C</td> <td>250</td> <td>250 of B</td> <td>50 ng/mL</td> </tr> <tr> <td>D</td> <td>250</td> <td>250 of C</td> <td>25 ng/mL</td> </tr> <tr> <td>E</td> <td>250</td> <td>250 of D</td> <td>12.5 ng/mL</td> </tr> <tr> <td>F</td> <td>250</td> <td>125 of E</td> <td>4.17 ng/mL</td> </tr> <tr> <td>G</td> <td>250</td> <td>125 of F</td> <td>1.39 ng/mL</td> </tr> <tr> <td>H</td> <td>250</td> <td>0</td> <td>0 ng/mL</td> </tr> </tbody> </table>	Assay standard	Sample buffer volume (µL)	Assay standard volume and source (µL)	Assay standard concentration	A	400	100 of stock	200 ng/mL	B	250	250 of A	100 ng/mL	C	250	250 of B	50 ng/mL	D	250	250 of C	25 ng/mL	E	250	250 of D	12.5 ng/mL	F	250	125 of E	4.17 ng/mL	G	250	125 of F	1.39 ng/mL	H	250	0	0 ng/mL
Assay standard	Sample buffer volume (µL)	Assay standard volume and source (µL)	Assay standard concentration																																		
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H	250	0	0 ng/mL																																		

Prepare the detection antibody working solution

Prepare the detection antibody working solution fresh for each assay, about 10 minutes before adding it to the plate.

Follow the steps below to prepare the detection antibody working solution.

Step	Action
1	Remove the vial from the refrigerator and allow it to reach room temperature (about 10 minutes).
2	Dilute HCPQuant CHO (Sup) Detection Antibody (detection antibody stock 120×) with the sample buffer. - For a complete 96-well plate, dilute 100 µL of the detection antibody stock with 12 mL of the sample buffer. - or - For a partial plate, use the table below to prepare the detection antibody working solution.

Table 5.2: Partial plate preparation table

Number of coated 1×8-well strips	2	3	4	5	6	7	8	9	10	11	12
Detection antibody stock volume (µL)	16	24	32	40	50	56	65	70	80	85	100
Sample buffer volume (mL)	1.8	2.9	3.8	4.8	6.0	6.7	7.8	8.4	9.6	10.2	12.0

Note:

Mix the detection antibody working solution by gently inverting the tube 3 to 5 times.

6 Sample preparation and matrix effect

Introduction

The performance of the kit was tested in various sample buffers (matrices) commonly used in the purification of biotherapeutics. Some buffer systems can significantly affect the assay, causing low recovery because of suboptimal conditions such as high salt concentrations or low pH.

Sample dilution

Optimal dilution factors for each test sample must be determined by the user. It is recommended to dilute the test sample first, to a minimum ratio of 1:1 using the HCPQuant CHO (Sup) Sample Buffer, and perform a serial dilution of the sample with HCPQuant CHO (Sup) Sample Buffer. For low pH buffers, a dilution of 1:10 might be required.

Note: *Remove insoluble protein aggregates by centrifuging the sample.*

Matrix effect

Validate specific matrix effects if the test sample is not diluted into HCPQuant CHO (Sup) Sample Buffer. Matrix effects can be quantified by spiking known concentrations of HCPQuant CHO (Sup) Protein Standard into test sample buffers. Matrix effects should be tested with different proportions of the HCPQuant CHO (Sup) Sample Buffer for optimal results. Recovery should be 80% to 120%.

7 Recommended assay procedure

Introduction

The assay procedure takes approximately 4 hours with a detection range of 1.39 to 200 ng/mL.

For a reproducible assay, we recommend the following:

- Mix the samples and reagents before use.
- Perform the washing procedure with the recommended wash buffer volumes.
- Measure all standards and samples in duplicate or triplicate.

Procedure

Before starting the procedure, let the buffer reagents and the plate (except the detection antibody) reach room temperature (20°C to 24°C).

Note: *This assay procedure was validated at a room temperature of approximately 22°C. The user is responsible for validating performance at temperatures lower than 20°C or higher than 24°C.*

Step	Action
1	Add 100 µL of each assay standard (see Table 5.1, on page 8) and 100 µL of the serial dilutions of the test samples to the designated wells. Note: <i>Use HCPQuant CHO (Sup) Sample Buffer to dilute the test samples. Suitable dilution factors should be determined empirically for each sample.</i>
2	Cover the plate using the included plate sealer and incubate at room temperature for 2 hours with continuous shaking (100 rpm).
3	Prepare the detection antibody working solution before the end of the incubation (see Prepare the detection antibody working solution, on page 9). Note: <i>Prepare the detection antibody working solution fresh for each assay, just before adding it to the plate.</i>
4	Wash the ELISA plate. • Wash the plate manually by following these instructions: a. Remove the plate sealer. b. Discard the well contents. c. Add 300 µL of wash buffer (1×) to each well and incubate for 10 seconds.

Step	Action
	d. Repeat steps a-c three more times. e. After the final wash, invert the plate and tap it against absorbent paper to remove any remaining wash buffer. or • Wash the plate using an automatic plate washer: a. Wash each well with 350 µL of wash buffer (1×). b. Incubate for 10 seconds between each wash. c. Repeat steps a-b three more times.
	Note: <i>Complete removal of the final wash buffer is critical for optimal assay performance.</i> Note: <i>Use a fresh plate sealer for each incubation to prevent contamination due to reuse.</i> Note: <i>Do not leave the wells without liquid for more than one minute. Add the next solution after complete removal of the wash buffer.</i>
5	Add 100 µL of the detection antibody working solution (1×) to each well, cover the plate, and incubate at room temperature for one hour with continuous shaking (100 rpm).
6	Wash the plate as described in step 4 . After the final wash, wipe the underside of the plate to remove residual liquid from the washing process.
7	Add 100 µL of TMB buffer to each well. Cover the plate while keeping it in the dark and incubate at room temperature for 30 minutes. Do not shake. Gently tap the plate periodically to mix.
8	Add 100 µL of stop buffer to each well to stop the reaction. Gently tap the plate to mix.
9	Read the plate using a spectrophotometer set at 450 nm wavelength with a reference wavelength at 630–650 nm.
	Note: <i>Read the plate within 30 minutes after the addition of stop buffer. The signal might be reduced with prolonged incubation.</i>

8 Calculations

Follow the steps below to estimate the CHO-HCP concentration of the test samples.

Step	Action
1	Calculate the relative OD 450 using the following formula: $\text{Relative OD 450} = (\text{OD 450 of well}) - (\text{OD of the blank well})$
2	Calculate the mean relative OD 450 of the replicates for each standard solution.
3	Plot the standard solutions data as mean relative OD 450 for each standard solution (Y) vs logarithm of the respective concentration of the standard solutions (X).
4	Fit the standard solution data with a four-parameter logistic (4-PL) curve.
5	Estimate the CHO-HCP concentration of the test samples using interpolation from the standard curve.

Note:

If the assay samples are from dilutions, multiply the concentrations obtained from interpolations by the dilution factor.

Note:

If the spectrometer used for the assay does not automatically subtract the reference wavelength, do this manually prior to subtracting the blank OD.

9 Assay performance characteristics

The complete validation package is available in the accompanying data file available on [cytiva.com](https://www.cytiva.com).

See the table below for general kit specifications and refer to the *Certificate of Analysis* for specific lot parameters.

Table 9.1: General kit specifications

Parameter	Specification
Goodness of fit of standard curve (4PL)	$R^2 > 0.98$
Range of the standard curve	1.39–200 ng/mL
Precision, intra- and inter-assay CV%	<20%
Sensitivity, lower limit of quantification	2 ng/mL
Sensitivity, lower limit of detection	<1.39 ng/mL

10 Troubleshooting

Issue	Possible solutions
Low reproducibility	• Do not mix components between kit lots. Each assay is lot-specific and designed for performance when used before the assigned expiration date. • Review washing step procedure. Add recommended volumes of wash buffer to all wells. • Review sample preparation and mixing procedures. Make sure that the samples are well-mixed and contain no protein aggregates. • Maintain appropriate reagent temperatures throughout the assay. • Liquid on the underside of the plate. Carefully dry the underside of the plate with an absorbent paper.
High background	• Review washing step procedure. Add recommended volumes of wash buffer to all wells. • Review the assay incubation procedure. Follow recommended incubation times. • Review sample preparation and mixing procedures. Make sure that the samples are well-mixed and contain no protein aggregates.
Poor reactivity or intra-assay precision	• Review sample preparation and mixing procedures. Make sure that the samples are well-mixed and contain no protein aggregates. • Review matrix effect: high concentrations of detergents, salts, pH and other matrices characteristics can influence the assay. • Use the provided sample buffer to dilute the sample to the minimum recommendations.
Poor fit of the standard curve	• Review washing step procedure. Add recommended volumes of wash buffer to all wells. • Review sample preparation and mixing procedures. Make sure that the samples are well-mixed and contain no protein aggregates. • Review pipetting procedure. Make sure that the samples are pipetted into the bottom of each well. Take care not to touch the bottom of the well with the pipette tip.

Appendix A

Quick Guide

Step	Task	Instruction
1	Bring the reagents and the plate to room temperature (20°C to 24°C).	Procedure, on page 11
2	Dilute wash buffer (10x) 1:10 with deionized water. Mix for at least 10 min.	Prepare the wash buffer (1×), on page 7
3	Reconstitute the protein standard. Prepare serial dilutions according to Table 5.1, on page 8 .	Prepare the assay standards, on page 8
4	Dilute samples at least 1:1 with sample buffer.	Sample dilution, on page 10
5	Add 100 µL of each sample and assay standard dilutions into designated wells.	Procedure, on page 11
6	Cover the plate and incubate at room temperature for 2 h with continuous shaking.	
7	Bring the detection antibody (120×) to room temperature. Dilute with sample buffer.	Prepare the detection antibody working solution, on page 9
8	Discard the well contents.	Procedure, on page 11
9	Wash the plate 4x with 300 µL of wash buffer (1×), soaking for 10 s between each wash. Discard the well contents after each wash. After the final wash, invert the plate and tap it against absorbent paper. Note: Do not leave the wells without liquid for more than one minute. Note: Alternatively, use an automated plate washer. Follow the instructions in step 4 on page 11.	
10	Add 100 µL of detection antibody working solution to each well.	
11	Cover the plate and incubate at room temperature for 1 h with continuous shaking.	
12	Wash the plate as described in step 9.	
13	Add 100 µL of TMB to each well and incubate in the dark for 30 minutes. Gently tap the plate periodically to mix.	
14	Add 100 µL of stop buffer to each well. Gently tap the plate to mix.	
15	Read the plate at 450 nm wavelength with a reference wavelength at 630–650 nm.	

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