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Product information

CHO HCP

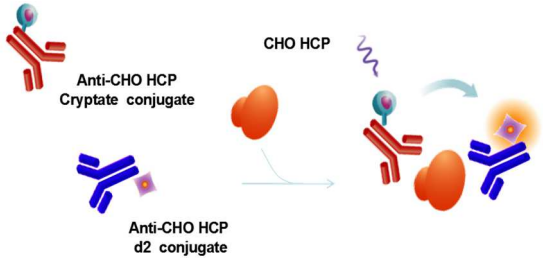
For in vitro research use only
Reagent storage temperature: -20°C or below

Packaging details :

	384-well low volume plate (20 µl)
6FHCPPEG	500 tests
Rev.02	

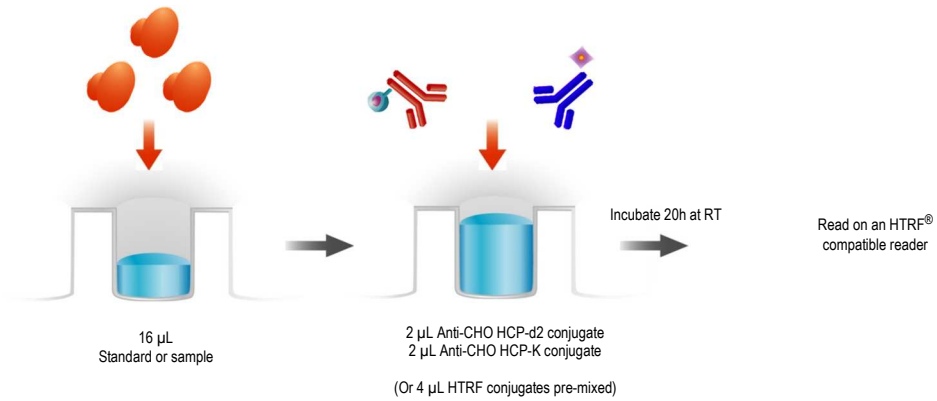
1. Assay description

This assay is intended for the quantitative determination of CHO HCP using the HTRF® technology. CHO HCP can be measured directly from cell supernatants or purified solutions.
As shown in the diagram to the right, CHO HCP is detected in a sandwich assay format using 2 specific antibodies, one labelled with Eu^{3+}-Cryptate (donor) and the second with d2 (acceptor).
When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665nm).



The two conjugates bind to the antigen present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen–antibody complexes formed and therefore to the CHO HCP concentration.

2. Protocol at a glance



3. HTRF® reagents

	CHO HCP Standard	Anti-CHO HCP Ab-d2 conjugate	Anti-CHO HCP Ab-Eu³⁺-Cryptate conjugate	Diluent 5X	Conjugate buffer
	 green cap	 blue cap	 red cap	 white cap	 orange cap
Stock solution	50 µL/vial 1500 ng/mL	25 µL/vial	25 µL/vial	2 mL/vial	2 x 2 mL/vial
Storage	-20°C or below	-20°C or below	-20°C or below	4°C to -20°C*	4°C to -20°C*
Ref # (when available separately)	6FHCPCDA	N/A	N/A	N/A	N/A

* Diluent and Conjugate buffer are shipped frozen, but can be stored at 2-8°C in your premises.

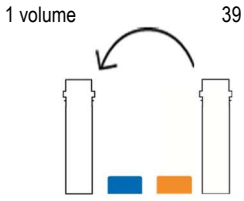
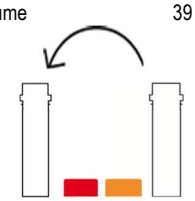
4. Reagent preparation

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate conjugates will impair the assay quality.
For an accurate quantitative determination of sample, dilution must be carried out with the medium used for preparing the samples (i.e. diluent, culture medium or any other compatible medium).
Standard and conjugates may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of standard and conjugates into disposable plastic vials for storage at –20°C or below.

- Thaw all reagents at room temperature.
- Prepare the working solutions from stock solutions (§3) by following the instructions below.

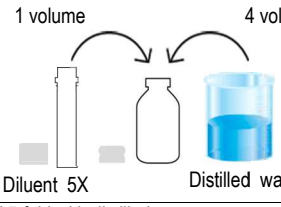
4.1. Preparation of conjugate working solutions

Determine the amount of conjugate needed for the experiment. Each well requires 2µL of each conjugate.

Anti-CHO HCP-d2 conjugate	Anti-CHO HCP-Eu³⁺-Cryptate conjugate
	
Prepare a 40X diluted solution using the conjugate buffer: e.g. take 25 µL of conjugate stock solution and add it to 975 µL of conjugate buffer.	Prepare a 40X diluted solution using the conjugate buffer: e.g. take 25 µL of conjugate stock solution and add it to 975 µL of conjugate buffer.

4.2. Preparation of diluent 1X

Prepare the required amount of diluent before running the assay.
Determine the amount of diluent needed for the experiment. Each well requires 16 µL of diluent. Prepare a diluent solution. In practice:



Dilute the "**diluent 5X**" 5-fold with distilled water to prepare diluent 1X.
e.g. take 1 mL of diluent 5X and add it to 4 mL of distilled water. Mix gently.

4.3. Standard curve preparation

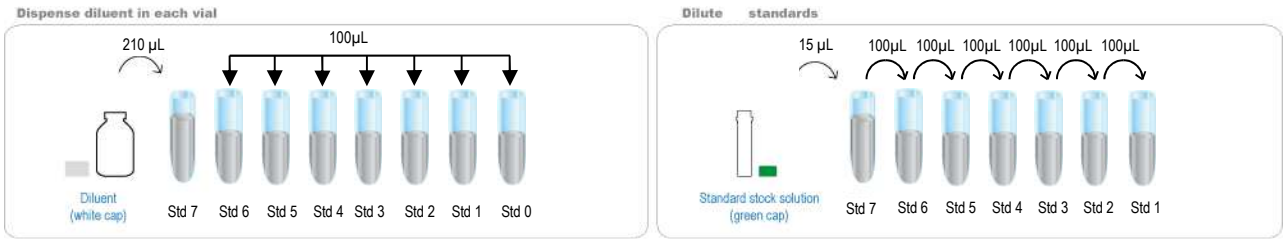
Determine how many samples and replicates to be tested. Each well requires 16 µL of sample or standard.
NB: If the sample to test is a cell supernatant, replace the diluent by culture medium.

Standards	Working concentration (ng/mL)	Preparation
Std 7	100	15 µL Standard stock solution + 210 µL diluent 1X
Std 6	50	100 µL Std 7 + 100 µL diluent 1X
Std 5	25	100 µL Std 6 + 100 µL diluent 1X
Std 4	12.5	100 µL Std 5 + 100 µL diluent 1X
Std 3	6.25	100 µL Std 4 + 100 µL diluent 1X
Std 2	3.13	100 µL Std 3 + 100 µL diluent 1X
Std 1	1.56	100 µL Std 2 + 100 µL diluent 1X
Std 0	0	100 µL diluent 1X

A recommended standard dilution procedure is listed and illustrated below.
→ *Dilute the standard stock solution 15-fold with diluent 1X; this yields the high standard (Std 7: 100 ng/mL) for the top of the curve.*
In practice:
- *e.g. take 15 µL of standard stock solution and add it to 210 µL of diluent 1X. Mix gently.*

→ *Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:*
- *Dispense 100 µL of diluent 1X in each vial from Std 6 to Std 1.*
- *Add 100 µL of standard to 100 µL of diluent 1X, mix gently and repeat the 1/2 serial dilution to make standard solutions: 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/mL.*

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent 1X alone.



5. Assay protocol

Dispense the reagents in the following order:

16 µL
Standard or sample

2 µL
Anti-CHO HCP-d2 conjugate

2 µL
Anti-CHO HCP-Eu^{3+}-Cryptate conjugate

Please Note: It is possible to pre-mix the two conjugates just before dispensing and add 4 µl of this mix.

→ Cover the plate with a plate sealer.
→ Incubate at RT for 20 hours.
→ Remove the plate sealer and,
→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

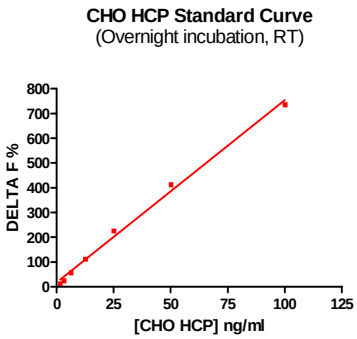
For more information about HTRF® compatible readers, please visit our website at: www.cisbio.com/readers

		Assay controls		
	Negative control	Cryptate control	Buffer control	Sample / Std
	Used to calculate the delta F %	*Used to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Sample / Std	-	-	-	16 µL
Diluent 1X	16 µL	16 µL	16 µL	-
Anti-CHO HCP-d2 conjugate	2 µL	-	-	2 µL
Anti-CHO HCP-Eu^{3+}-Cryptate conjugate	2 µL	2 µL	-	2 µL
Conjugate buffer	-	2 µL	4 µL	-

6. Data reduction

These data must not be substituted for that obtained in the laboratory and should be considered only as an example (readouts on PHERAstar^{FS}). Results may vary from one HTRF® compatible reader to another.
The assay standard curve is drawn up by plotting delta F% versus the analyte concentration:

Standards ng / mL	Ratio (1)	CV % (2)	Delta F % (3)
Std 0 - Negative control	497	3.0%	0
Std 1 - 1.56	561	1.5%	13
Std 2 - 3.13	618	3.9%	25
Std 3 - 6.25	778	2.5%	57
Std 4 - 12.5	1059	3.6%	113
Std 5 - 25	1621	1.9%	226
Std 6 - 50	2547	1.5%	413
Std 7 - 100	4153	4.1%	736



Ratio (1)	$\frac{\text{Signal}_{665nm}}{\text{Signal}_{620nm}} \times 10^4$	Ratio must be calculated for each individual well.
CV% (2)	$\frac{\text{Standard deviation}}{\text{Mean ratio}} \times 100$	The mean and standard deviation can then be worked out from ratio replicates.
Delta F % (3)	$\frac{\text{Ratio}_{\text{standard or sample}} - \text{Ratio}_{\text{Negative control}}}{\text{Ratio}_{\text{Negative control}}} \times 100$	Reflects the signal to background of the assay. The negative control plays the role of an internal assay control.
For more information about data reduction, please visit our website at: www.cisbio.com/data-reduction		

To obtain additional information or support, please contact your technical support team (htrfservices@cisbio.com).