MANUAL

HTRF CHO HCP DETECTION KITS

Part # 64CHOPEG & 64CHOPEH

Test Size#: 500 TESTS (64CHOPEG), 10,000 TESTS(64CHOPEH)

Revision: #02 of September 2023 **Store at:** ≤-16°C

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Chinese Hamster Ovary (CHO) cells Host Cell Proteins (HCP) and offers a fast alternative to ELISA.

HCP are process-related protein impurities found in drug product derived from host organisms (bacterial, yeast or mammalian production cell lines) during biotherapeutic manufacturing and purification. Among protein expression cell lines, the most commonly used mammalian hosts for industrial production of recombinant protein therapeutics are CHO cells. During expression of a recombinant protein drug, CHO cells can express many endogenous proteins, called HCP. Despite downstream processing of biopharmaceuticals remove most of these HCP contaminants, there are concerns about the presence of residual HCP in the final product due to potential adverse clinical effects, decrease in drug product efficacy and stability. Hence, detection and quantification of HCP impurities is critical for biopharmaceutical companies in agreement with regulatory agency guidelines.

HTRF CHO HCP Detection Kits are designed to quantitatively measure CHO HCP contaminations in routine bioprocess operations using CHO expression systems, from the very crude harvest material to the final product.

The detection principle is based on HTRF® (Homogeneous Time-Resolved Fluorescence) technology. As shown in Figure 1, CHO HCP are detected in a sandwich assay format using a qualified anti-CHO HCP polyclonal antibodies pool, labeled with Europium Cryptate (donor) and 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 (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the HCP CHO concentration.

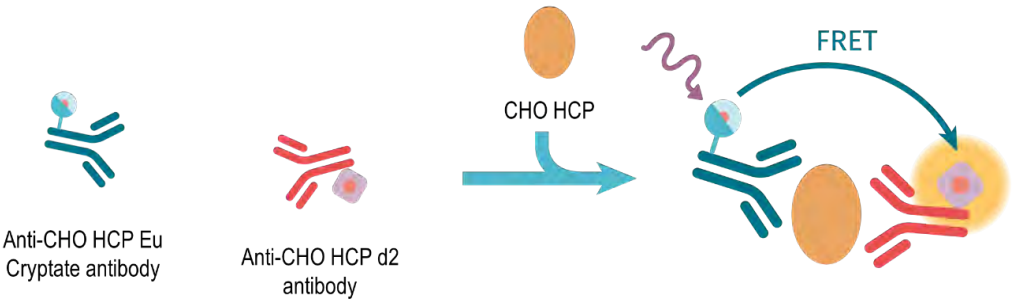
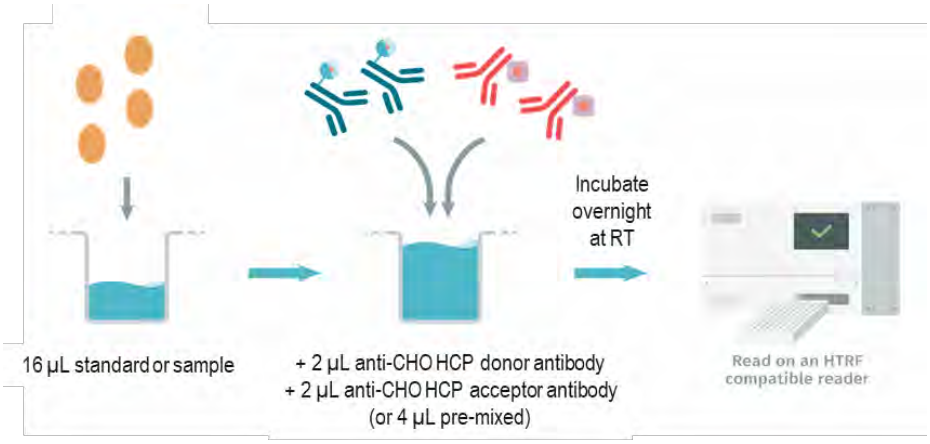


Figure 1: Principle of HTRF CHO HCP sandwich assay

MANUAL AT A GLANCE



MATERIALS PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS* CAT# 64CHOPEG		10,000 TESTS* CAT# 64CHOPEH	
CHO HCP Eu Cryptate Antibody (stock solution 20X)	≤-16°C	Orange cap	1 vial - 50 µL	Red cap	1 vial – 1 mL
CHO HCP d2 Antibody (stock solution 20X)	≤-16°C	Blue cap	1 vial - 50 µL	Purple cap	1 vial – 1 mL
Lyophilized CHO HCP Standard	2-8°C	Green cap	1 vial 1,200 ng/mL	Green cap	2 vials 1,200 ng/mL
Diluent #5** (stock solution 5X)	≤-16°C	Yellow cap	1 vial – 2 mL	White cap	1 vial – 10 mL
Detection buffer #17 ** (ready-to-use)	≤-16°C	Transparent cap	2 vials – 2 mL	Red cap	1 vial – 50 mL

*When used as advised, the two available kit sizes will provide sufficient reagents for 500 and 10,000 tests respectively in 20 µL final.
Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

► PURCHASE SEPARATELY

*HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.
For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

*Small volume (SV) detection microplates.
For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Storage upon reception:
Store the kit at -16°C or below until the expiration date indicated on the package.

Storage and stability of thawed material:
When you are ready to use the kit, take the reagents out and prepare them following the manual provided in this document. Unused thawed reagents can be stored and conserved for future use. Refer to the table below for storage options and corresponding shelf life.

	Storage after Thawing/reconstitution
Detection buffer	2-8°C until the expiration date indicated on the package
Antibodies*	2-8°C for 48h or freeze at -16°C or below until the expiration date indicated on the package for long term storage
Lyophilized standard**	Freeze at -16°C or below until the expiration date indicated on the package for long term storage

*For Antibodies, stock solutions may be thawed and frozen only once. Freeze in aliquots to avoid multiple freeze/thaw cycles (once aliquoted, single use of the reagent). Volume of antibodies aliquots should not be under 10µL.
*For Lyophilized standard, after reconstitution, to avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -16°C or below. This standard stock solution may be frozen and thawed only once. Volume of CHO HCP standard aliquots should not be under 20 µL.

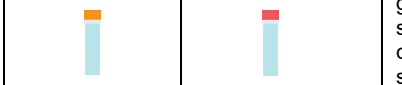
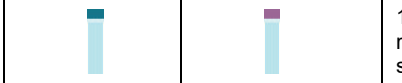
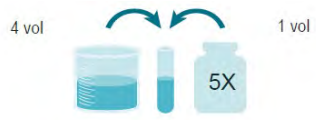
REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Avoid freeze thawing cycles.
- Thaw the frozen reagents at room temperature, allow them to warm up at room temperature at least 30 minutes before use.
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- CHO HCP standard (for standard curve) must be prepared in Diluent #5 or in the same medium as the samples. Some components used for biotherapeutics manufacturing may yield to slight interference in the assay. So, to reduce possible matrice effects, it is advised to dilute samples containing high HCP concentration in Diluent #5. When testing samples without dilution, it is recommended to compare standard curves in both Diluent #5 and sample's buffer. If the sample's buffer impairs standard curve results, we recommend to run it in sample buffer instead of Diluent #5.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

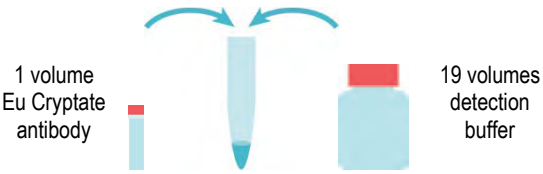
500 TESTS KIT CAT# 64CHOPEG		10,000 TESTS KIT CAT# 64CHOPEH
CHO HCP Eu cryptate antibody		
Thaw the CHO HCP Eu Cryptate antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.		Thaw the CHO HCP Eu Cryptate antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
CHO HCP d2 antibody		
Thaw the CHO HCP d2 antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.		Thaw the CHO HCP d2 antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
CHO HCP Standard		
Reconstitute the CHO HCP Standard with distilled water in order to obtain a 1,200 ng/ mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. After use, the reconstituted standard solution must be frozen and stored at -16°C or below.		Reconstitute the CHO HCP Standard with distilled water in order to obtain a 1,200 ng/ mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. After use, the reconstituted standard solution must be frozen and stored at -16°C or below.
Diluent		
Dilute 5-fold the 5X Diluent #5 with distilled water: Homogenize the 5X Diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g. 1 mL of diluent + 4 mL of distilled water). Mix gently after dilution. This 1X solution can be frozen and stored at -16°C or below.		Dilute 5-fold the 5X Diluent #5 with distilled water: Homogenize the 5X Diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g. 1 mL of diluent + 4 mL of distilled water). Mix gently after dilution. This 1X solution can be frozen and stored at -16°C or below.
Detection buffer		
The detection buffer is ready-to-use.		The detection buffer is ready-to-use.

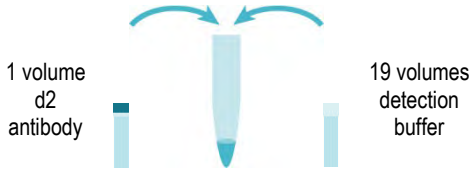
TO PREPARE WORKING ANTIBODY SOLUTIONS:

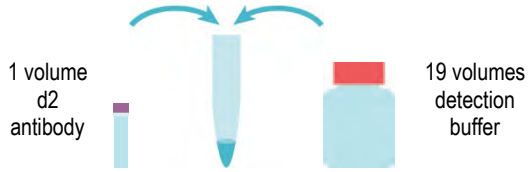
Each well requires 2µL of CHO HCP-Eu Cryptate Antibody and 2µL of CHO HCP-d2 Antibody.

Prepare the two antibody solutions in separate vials.

<table>
<tr><th>500 TESTS KIT CAT# 64CHOPEG</th><th>10,000 TESTS KIT CAT# 64CHOPEH</th></tr>
<tr><td colspan="2">CHO HCP-Eu cryptate antibody</td></tr>
<tr><td> 

Dilute 20-fold the 20X frozen stock solution of CHO HCP Eu Cryptate antibody with detection buffer, e.g. add 950 µL of detection buffer to 50 µL of Eu Cryptate -antibody stock solution.</td><td> 

Dilute 20-fold the 20X frozen stock solution of CHO HCP Eu antibody with detection buffer, e.g. add 950 µL of detection buffer to 50 µL of Eu Cryptate -antibody stock solution.</td></tr>
<tr><td colspan="2">CHO HCP-d2 antibody</td></tr>
<tr><td> 

Dilute 20-fold the 20X frozen stock solution of CHO HCP d2 antibody with detection buffer, e.g. add 950 µL of detection buffer to 50 µL of d2-antibody stock solution.</td><td> 

Dilute 20-fold the 20X frozen stock solution of CHO HCP d2 antibody with detection buffer, e.g. add 950 µL of detection buffer to 50 µL of d2-antibody stock solution.</td></tr>
<tr><td colspan="2">Antibody mix</td></tr>
<tr><td>It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Eu Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Eu Cryptate antibody).</td><td>  </td><td>It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Eu Cryptate antibody solution (e.g. 20 mL of d2 antibody + 20 mL of Eu Cryptate antibody).</td></tr>
</table>

TO PREPARE STANDARD WORKING SOLUTIONS:

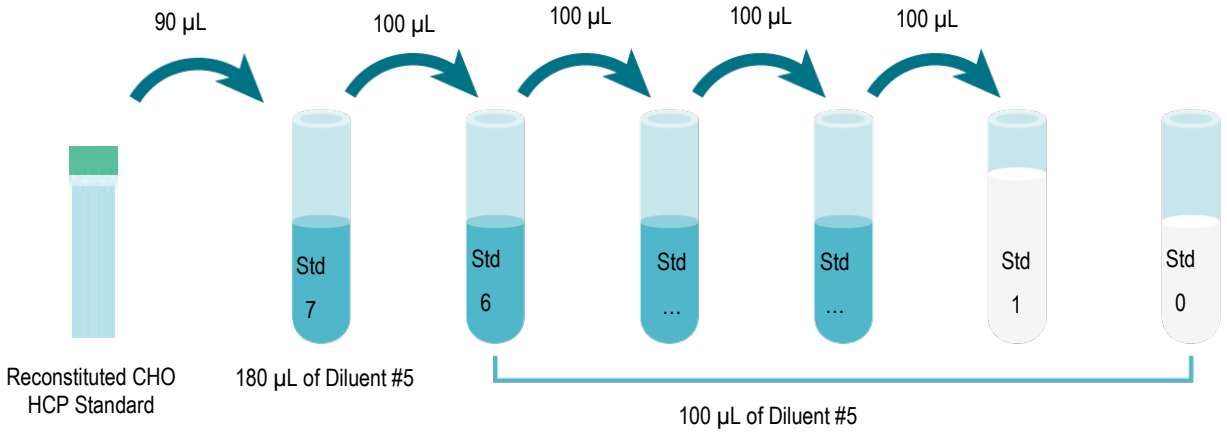
- Each well requires 16µL of standard.
- Dilute the standard stock solution serially with Diluent #5 (1X) or in the medium used for the preparation of the samples.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own assay buffer and in Diluent #5 (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

Dilution the standard stock solution 3-fold with Diluent #5 (1X) to prepare the high standard (Std 7, 1,200 ng/mL): e.g. take 90 µL of standard stock solution and add it to 180 µL of Diluent #5 (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 #5 (1X) in each vial from Std 6 to Std 0.
- Add 100 µL of standard 7 to 100 µL of Diluent #5 (1X), mix gently and repeat the 1/2 serial dilution to make standard solutions: Std 6, Std 5, Std 4, Std 2 and Std 1.
- This will create 7 standards for the analyte. Std 0 (Negative control) is Diluent #5 (1X).



STANDARD	SERIAL DILUTIONS	CHO HCP WORKING CONCENTRATION (ng/mL)
Standard Stock solution	Reconstituted lyophilizate	1,200
Standard 7	90 µL of standard stock solution + 180 µL Diluent #5 (1X)	400
Standard 6	100 µL standard 7 +100 µL Diluent #5 (1X)	200
Standard 5	100 µL standard 6 +100 µL Diluent #5 (1X)	100
Standard 4	100 µL standard 5 +100 µL Diluent #5 (1X)	50
Standard 3	100 µL standard 4 +100 µL Diluent #5 (1X)	25
Standard 2	100 µL standard 3 +100 µL Diluent #5 (1X)	12.5
Standard 1	100 µL standard 2 +100 µL Diluent #5 (1X)	6.25
Standard 0	100 µL Diluent #5 (1X)	0

TO PREPARE SAMPLES:

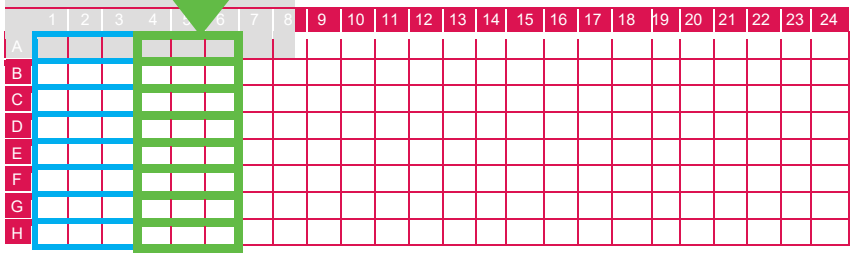
- Each well requires 16µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted in Diluent #5 (1X) prepared, as recommended above.
- To obtain additional information or support, please contact the HTRF technical support team at Revvity.com/contact us.

ASSAY MANUAL

	STANDARD (STD 0 – STD 7)	SAMPLES
Step 1 	Dispense 16 µL of each CHO HCP standard (Std 0 – Std 7) into each standard well	Dispense 16 µL of each sample into each sample well
Step 2 	Add 2 µL of CHO HCP d2 antibody working solution to all wells*	
Step 3 	Add 2 µL of CHO HCP Eu Cryptate antibody working solution to all wells*	
Step 4 	Seal the plate and incubate ON @ RT Following incubation, the signal remains stable over a period of 48 hours.	
Step 5 	Remove the plate sealer and read on an HTRF® compatible reader	

* As indicated in the preparation of working antibody solutions, it is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Eu Cryptate antibody solution. In this case, Step 2 and Step 3 are replaced by one Step: add 4 µL of pre-mixed CHO HCP antibodies working solution to all wells.

	1	2	3	4	5	6
A	16 µL Std 0 (negative control) 4 µL pre-mixed CHO HCP antibodies	Repeat Well A1	Repeat Well A1	16 µL Sample 1 4 µL pre-mixed CHO HCP antibodies	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 4 µL pre-mixed CHO HCP antibodies	Repeat Well B1	Repeat Well B1	16 µL Sample 2 4 µL pre-mixed CHO HCP antibodies	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 4 µL pre-mixed CHO HCP antibodies	Repeat Well C1	Repeat Well C1	16 µL Sample 3 4 µL pre-mixed CHO HCP antibodies	Repeat Well C4	Repeat Well C4
D	16 µL Std 3… 4 µL pre-mixed CHO HCP antibodies	Repeat Well D1	Repeat Well D1	16 µL Sample 4 4 µL pre-mixed CHO HCP antibodies	Repeat Well D4	Repeat Well D4
E	16 µL Std 4… 4 µL pre-mixed CHO HCP antibodies	Repeat Well E1	Repeat Well E1	16 µL Sample 5 4 µL pre-mixed CHO HCP antibodies	Repeat Well E4	Repeat Well E4
F	16 µL Std 5… 4 µL pre-mixed CHO HCP antibodies	Repeat Well F1	Repeat Well F1	16 µL Sample 6 4 µL pre-mixed CHO HCP antibodies	Repeat Well F4	Repeat Well F4
G	16 µL Std 6… 4 µL pre-mixed CHO HCP antibodies	Repeat Well G1	Repeat Well G1	16 µL Sample 7 4 µL pre-mixed CHO HCP antibodies	Repeat Well G4	Repeat Well G4
H	16 µL Std 7… 4 µL pre-mixed CHO HCP antibodies	Repeat Well H1	Repeat Well H1	16 µL Sample 8 4 µL pre-mixed CHO HCP antibodies	Repeat Well H4	Repeat Well H4



DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the Delta Ratio for each sample. The Standard 0 (Negative control) plays the role of an internal assay control.

Delta Ratio = Ratio Sample or standard - Ratio Negative Control (Standard 0)

For more information about data reduction, please visit www.revvity.com

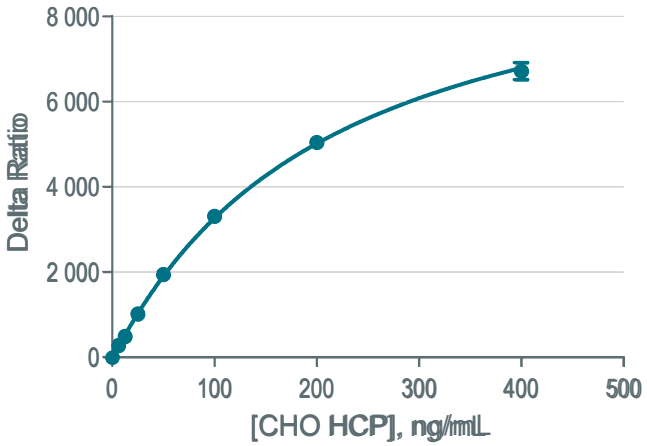
RESULTS

These data should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL) model (with 1/Y² weighting):

Standard	CHO HCP concentration (ng/mL)	Mean HTRF Ratio	CV %	Delta Ratio
Standard 0	0	773	2%	0
Standard 1	6.25	1050	1%	277
Standard 2	12.5	1260	1%	487
Standard 3	25	1793	2%	1020
Standard 4	50	2717	5%	1944
Standard 5	100	4084	1%	3311
Standard 6	200	5813	2%	5040
Standard 7	400	7486	3%	6713

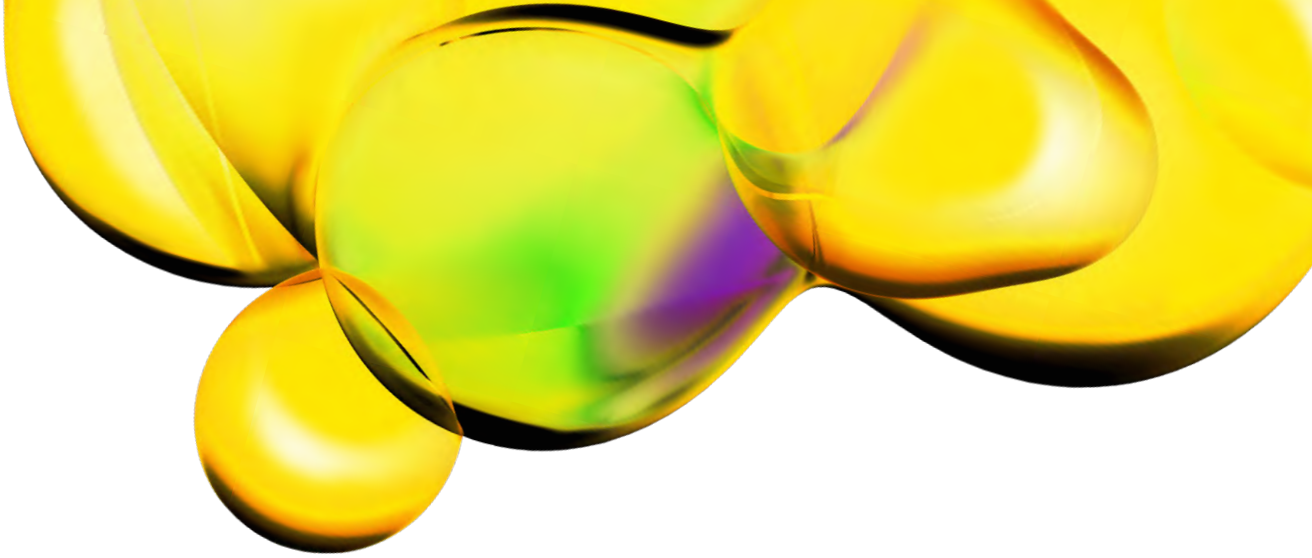


ANALYTICAL CHARACTERISTICS

ASSAY PERFORMANCES

Assay range (Limit of Quantification (LOQ)* to Std max)	3.6 - 400 ng/mL
Limit Of Detection (LOD)* = Mean Std 0 + 2 SD	0.8 ng/ml
Incubation time	Overnight at room temperature

**The LOD and LOQ may vary from one HTRF compatible reader to another.*



Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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