MANUAL

HUMAN C1Q BINDING KITS

Part # 64C1QPET & 64C1QPEG Biomarkers

Test Size#: 100 TESTS (64C1QPET), 500 TESTS (64C1QPEG) - assay volume: 20 µL

Revision: #02 of September 2023 **Store at:** ≤-60°C

This product is intended for research purposes only. It is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

This kit is intended for measuring the binding of the Fc region of IgG antibodies to human complement component C1q in buffered solution. Unlike conventional methods that require the immobilization of antibodies on a solid phase, this assay enables simple and rapid characterization of antibodies in a homogeneous generic format. The detection principle is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, an anti-human IgG Fab-biotin complexed to streptavidin is used to capture and aggregate the tested antibody in solution. Antibodies bound to human C1q are detected in a sandwich assay format using an anti-C1q antibody (anti-collagen-like region) labelled with Europium cryptate (donor), and the streptavidin labelled 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 (665 nm). Signal intensity is directly proportional to the capacity of the antibody Fc region to interact with human C1q.

The assay is compatible with chimeric, humanized and fully human antibodies, as well as with IgG1, IgG2 and IgG4 isotypes.

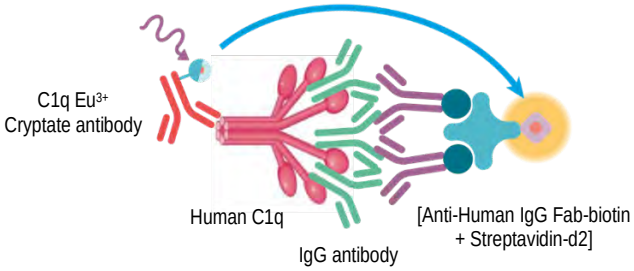
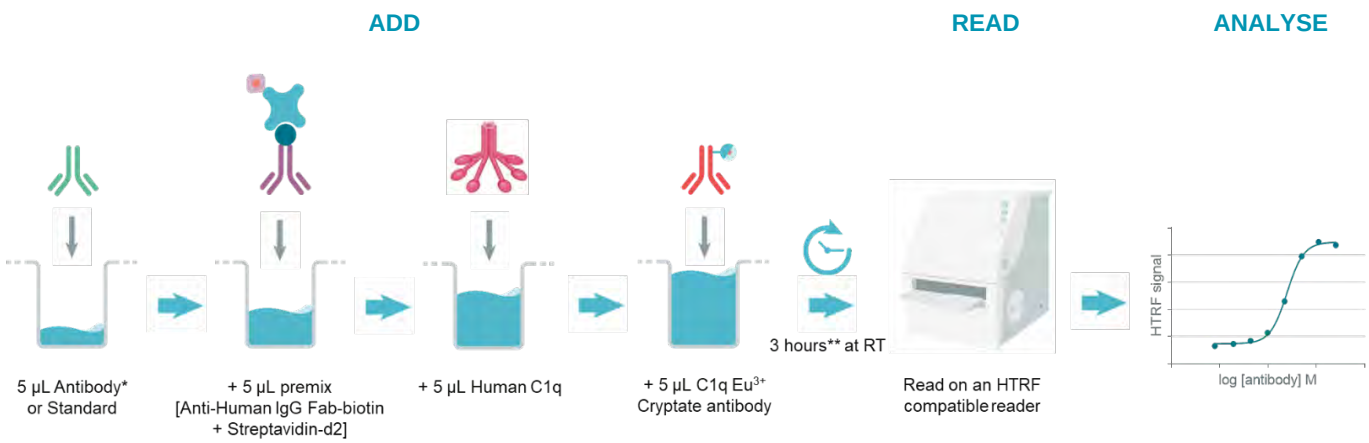


Figure 1: Principle of HTRF Human C1q Binding assay

MANUAL AT A GLANCE



All assay components (tested antibodies and kit reagents) must be diluted with kit diluent.
* The tested antibody must be in a purified format (known concentration).
** Following incubation, the signal remains stable over a period of 48 hours.

Make sure you use the appropriate setup for Eu^{3+} Cryptate. For more information about setup and HTRF® compatible readers, please visit our website at: www.revvity.com

MATERIALS PROVIDED:

KIT COMPONENTS	100 TESTS* CAT# 64C1QPET	500 TESTS* CAT# 64C1QPEG
HTRF Human C1q Binding Kit - Standard # 64C1QCDA	1 vial - 200 µL Frozen - 5X	1 vial - 200 µL Frozen - 5X
Anti-Human IgG Fab-biotin	1 vial - 100 µL Frozen - 5X	1 vial - 500 µL Frozen - 5X
Streptavidin-d2	1 vial - 100 µL Frozen - 5X	1 vial - 500 µL Frozen - 5X
C1q Eu Cryptate antibody	1 vial - 20 µL Frozen - 25X	1 vial - 100 µL Frozen - 25X
Human C1q	1 vial - 20 µL Frozen - 25X	1 vial - 100 µL Frozen - 25X
Diluent #12**	1 vial - 20 mL Ready-to-use	1 vial - 100 mL Ready-to-use

* When used as advised, the two available kit sizes will provide sufficient reagents for 100 and 500 tests respectively in 20 µL final.
Assay volumes can be adjusted proportionally to run the assay in 96- or 1536-well microplates.
** Diluent #12 is used to prepare working solutions of kit reagents and tested antibodies.

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

• Low volume detection microplates. Use white plates only.
For more information about microplate recommendations, please visit www.revvity.com

STORAGE AND STABILITY

Store the kit at ≤-60°C. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Stock solutions of Standard, C1q Eu Cryptate antibody, Streptavidin-d2, Anti-Human IgG Fab-biotin, and Human C1q can be frozen and thawed only twice. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at the recommended temperature (≤-60°C for Human C1q or ≤-16°C for the other reagents). Volume of reagent aliquots should not be under 10 µL.

Thawed Diluent #12 can be stored at 2-8°C on your premises.

REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare all reagents in Diluent #12. The use of an incorrect diluent may affect reagent stability and assay results.
- Before use, allow Diluent #12 to warm up at room temperature and homogenize it with a vortex.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 10 min before use.
- We recommend centrifuging the vials gently after thawing, before pipetting the stock solutions.

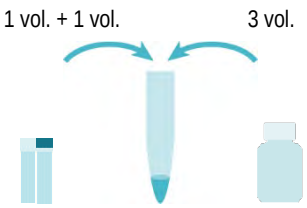
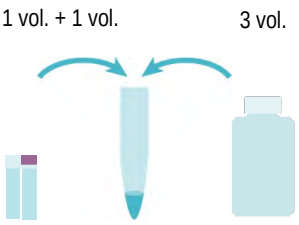
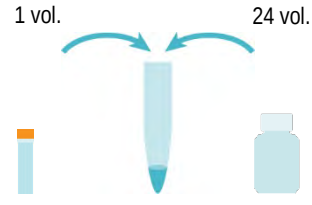
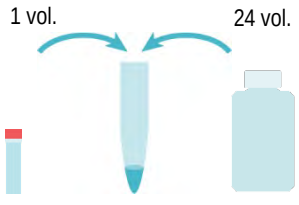
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

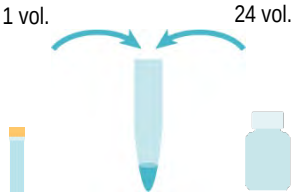
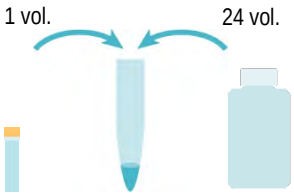
TO PREPARE STOCK SOLUTIONS:

100 TESTS KIT - 64C1QPET		500 TESTS KIT - 64C1QPEG	
HTRF Human C1q Binding Kit - Standard			
Thaw the HTRF Human C1q Binding Kit - Standard. Mix gently. This 5X stock solution (1.4 µM) can be frozen and stored at ≤ -16°C.			Thaw the HTRF Human C1q Binding Kit - Standard. Mix gently. This 5X stock solution (1.4 µM) can be frozen and stored at ≤ -16°C.
Anti-Human IgG Fab-biotin			
Thaw the Anti-Human IgG Fab-biotin. Mix gently. This 5X stock solution can be frozen and stored at ≤ -16°C.			Thaw the Anti-Human IgG Fab-biotin. Mix gently. This 5X stock solution can be frozen and stored at ≤ -16°C.
Streptavidin-d2			
Thaw the Streptavidin-d2. Mix gently. This 5X stock solution can be frozen and stored at ≤ -16°C.			Thaw the Streptavidin-d2. Mix gently. This 5X stock solution can be frozen and stored at ≤ -16°C.
C1q Eu Cryptate antibody			
Thaw the C1q Eu Cryptate antibody. Mix gently. This 25X stock solution can be frozen and stored at ≤ -16°C.			Thaw the C1q Eu Cryptate antibody. Mix gently. This 25X stock solution can be frozen and stored at ≤ -16°C.
Human C1q			
Thaw the Human C1q. Mix gently. This 25X stock solution can be frozen and stored at ≤ -60°C.			Thaw the Human C1q. Mix gently. This 25X stock solution can be frozen and stored at ≤ -60°C.
Diluent #12			
Thaw Diluent #12. Mix gently. This 1X ready-to-use buffer can be stored at 2-8°C.			Thaw Diluent #12. Mix gently. This 1X ready-to-use buffer can be stored at 2-8°C.

TO PREPARE REAGENT WORKING SOLUTIONS:

Each well requires 5 µL of each reagent. Prepare in separate vials.

100 TESTS KIT - 64C1QPET		500 TESTS KIT - 64C1QPEG	
Premix [Anti-Human IgG Fab-biotin + Streptavidin-d2]			
Dilute 5-fold the 5X stock solutions (thawed reagents) of Anti-Human IgG Fab-biotin and Streptavidin-d2 with Diluent #12: add 1 volume of Anti-Human IgG Fab-biotin stock solution and 1 volume of Streptavidin-d2 stock solution in 3 volumes of Diluent #12 (e.g. 100 µL of Anti-Human IgG Fab-biotin stock solution + 100 µL of Streptavidin-d2 stock solution + 300 µL of Diluent #12). It is mandatory to prepare this premix just prior dispensing.			Dilute 5-fold the 5X stock solutions (thawed reagents) of Anti-Human IgG Fab-biotin and Streptavidin-d2 with Diluent #12: add 1 volume of Anti-Human IgG Fab-biotin stock solution and 1 volume of Streptavidin-d2 stock solution in 3 volumes of Diluent #12 (e.g. 500 µL of Anti-Human IgG Fab-biotin stock solution + 500 µL of Streptavidin-d2 stock solution + 1.5 mL of Diluent #12). It is mandatory to prepare this premix just prior dispensing.
C1q Eu Cryptate antibody			
Dilute 25-fold the 25X stock solution (thawed reagent) of C1q Eu Cryptate antibody with Diluent #12: add 1 volume of C1q Eu Cryptate antibody stock solution in 24 volumes of Diluent #12 (e.g. 20 µL of C1q Eu Cryptate antibody stock solution + 480 µL of Diluent #12).			Dilute 25-fold the 25X stock solution (thawed reagent) of C1q Eu Cryptate antibody with Diluent #12: add 1 volume of C1q Eu Cryptate antibody stock solution in 24 volumes of Diluent #12 (e.g. 100 µL of C1q Eu Cryptate antibody stock solution + 2.4 mL of Diluent #12).

Human C1q			
Dilute 25-fold the 25X stock solution (thawed reagent) of Human C1q with Diluent #12: add 1 volume of Human C1q stock solution in 24 volumes of Diluent #12 (e.g. 20 µL of Human C1q stock solution + 480 µL of Diluent #12).			Dilute 25-fold the 25X stock solution (thawed reagent) of Human C1q with Diluent #12: add 1 volume of Human C1q stock solution in 24 volumes of Diluent #12 (e.g. 100 µL of Human C1q stock solution + 2.4 mL of Diluent #12).

TO PREPARE STANDARD WORKING SOLUTIONS:

The standard provided in the kit is a purified human IgG1 recombinant antibody. One vial is sufficient to perform 5 standard curves.

- Each well requires 5 µL of standard solution.
- Dilute the standard stock solution serially with Diluent #12.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

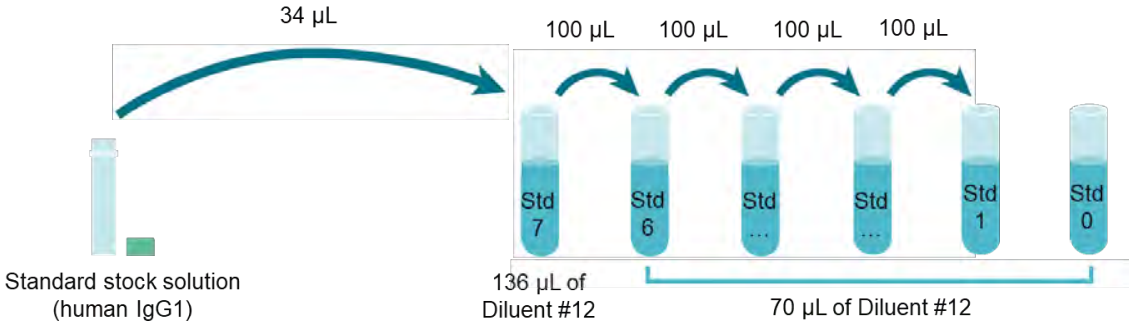
Dilute the standard stock solution 5-fold with Diluent #12 to prepare high standard (Std 7): e.g. take 34 µL of standard stock solution (1.4 µM) and add it to 136 µL of Diluent #12. Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/1.7 serial dilutions as follows:

- Dispense 70 µL of Diluent #12 in each vial from Std 6 to Std 0.
- Add 100 µL of standard 7 to 70 µL of Diluent #12, mix gently and repeat the 1/1.7 serial dilutions to make standard solutions:

Std6, Std 5, Std 4, Std 3, Std 2, Std 1.

This will create 7 standards for the human IgG1 standard. Std 0 (Negative control) is Diluent #12 alone.



STANDARD	PREPARATION	STANDARD WORKING CONCENTRATION (4X)	STANDARD FINAL CONCENTRATION (1X)
Standard Stock solution	Thawed stock solution (1.4 µM)	-	-
Standard 7	34 µL standard stock solution + 136 µL Diluent #12	280 nM	70 nM
Standard 6	100 µL Standard 7 + 70 µL Diluent #12	164.7 nM	41.2 nM
Standard 5	100 µL Standard 6 + 70 µL Diluent #12	96.9 nM	24.2 nM
Standard 4	100 µL Standard 5 + 70 µL Diluent #12	57.0 nM	14.3 nM
Standard 3	100 µL Standard 4 + 70 µL Diluent #12	33.5 nM	8.4 nM
Standard 2	100 µL Standard 3 + 70 µL Diluent #12	19.7 nM	4.9 nM
Standard 1	100 µL Standard 2 + 70 µL Diluent #12	11.6 nM	2.9 nM
Standard 0 (Negative control)	70 µL Diluent #12	0	0

TO PREPARE TESTED ANTIBODY WORKING SOLUTIONS:

The assay is compatible with chimeric, humanized and fully human antibodies, as well as with IgG1, IgG2 and IgG4 isotypes.

The tested antibodies must be in a purified format (known concentration). They should be dispensed into disposable plastic vials and stored at the appropriate temperature.

- Each well requires 5 µL of tested antibody.
- Dilute the tested antibody stock solution serially with Diluent #12.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

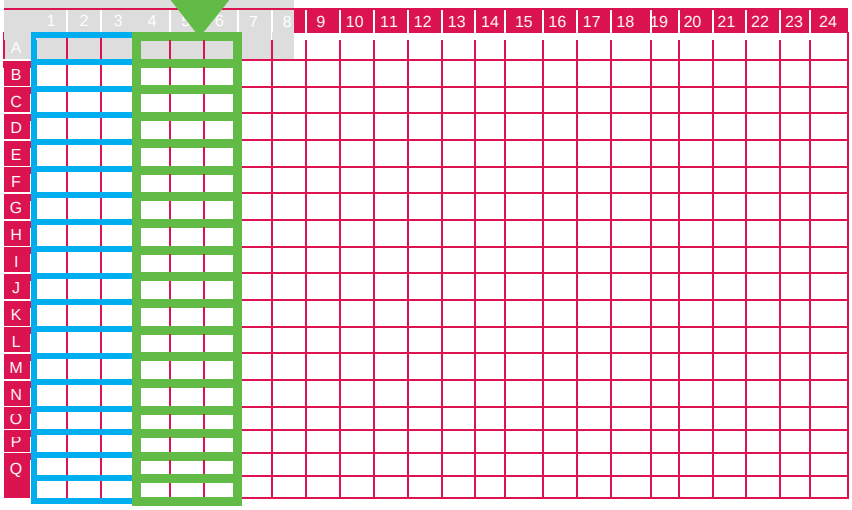
ASSAY MANUAL

	NEGATIVE CONTROL	STANDARD	TESTED ANTIBODIES
Step 1 	Dispense 5 µL of Diluent #12 (Std 0) into each negative control well	Dispense 5 µL of each standard solution (Std 1 - Std 7) into each standard well	Dispense 5 µL of each tested antibody solution into each test well
Step 2 	Add to all wells, in the following order: 5 µL of Premix [Anti-Human IgG Fab-biotin + Streptavidin-d2], 5 µL of Human C1q, and 5 µL of C1q Eu Cryptate antibody		
Step 3 	Seal the plate and incubate for 3 hours at RT Following incubation, the signal remains stable over a period of 48 hours		
Step 4 	Remove the plate sealer and read on an HTRF® compatible reader		

EXAMPLE OF PLATE MAP:

	1	2	3	4	5	6
A	***Negative control*** 5 µL Diluent #12 (Standard 0) + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well A1	Repeat Well A1	***Tested antibody solution 1*** 5 µL tested antibody solution 1 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well A4	Repeat Well A4
B	***Standard 1*** 5 µL Standard 1 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well B1	Repeat Well B1	***Tested antibody solution 2*** 5 µL tested antibody solution 2 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well B4	Repeat Well B4
C	***Standard 2*** 5 µL Standard 2 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well C1	Repeat Well C1	***Tested antibody solution ...*** 5 µL tested antibody solution ... + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well C4	Repeat Well C4
D	***Standard 3*** 5 µL Standard 3 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well D1	Repeat Well D1	***Tested antibody solution ...*** 5 µL tested antibody solution ... + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well D4	Repeat Well D4
E	***Standard 4*** 5 µL Standard 4 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well E1	Repeat Well E1	***Tested antibody solution ...*** 5 µL tested antibody solution ... + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well E4	Repeat Well E4
F	***Standard 5*** 5 µL Standard 5 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well F1	Repeat Well F1	***Tested antibody solution ...*** 5 µL tested antibody solution ... + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well F4	Repeat Well F4
G	***Standard 6*** 5 µL Standard 6 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well G1	Repeat Well G1	***Tested antibody solution ...*** 5 µL tested antibody solution ... + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well G4	Repeat Well G4

H	***Standard 7*** 5 µL Standard 7 + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	Repeat Well H1	Repeat Well H1	***Tested antibody solution ...*** 5 µL tested antibody solution ... + 5 µL premix [Anti-Human IgG Fab-biotin + Streptavidin-d2] + 5 µL Human C1q + 5 µL C1q Eu Cryptate antibody	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.

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

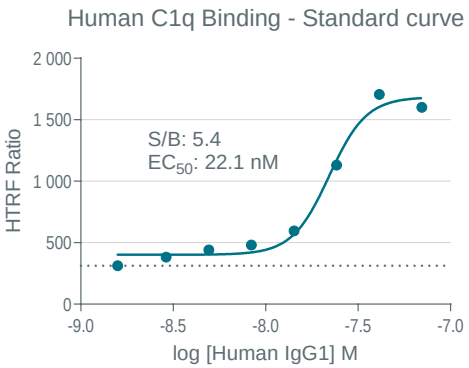
For more information about data reduction, please visit www.revvity.com

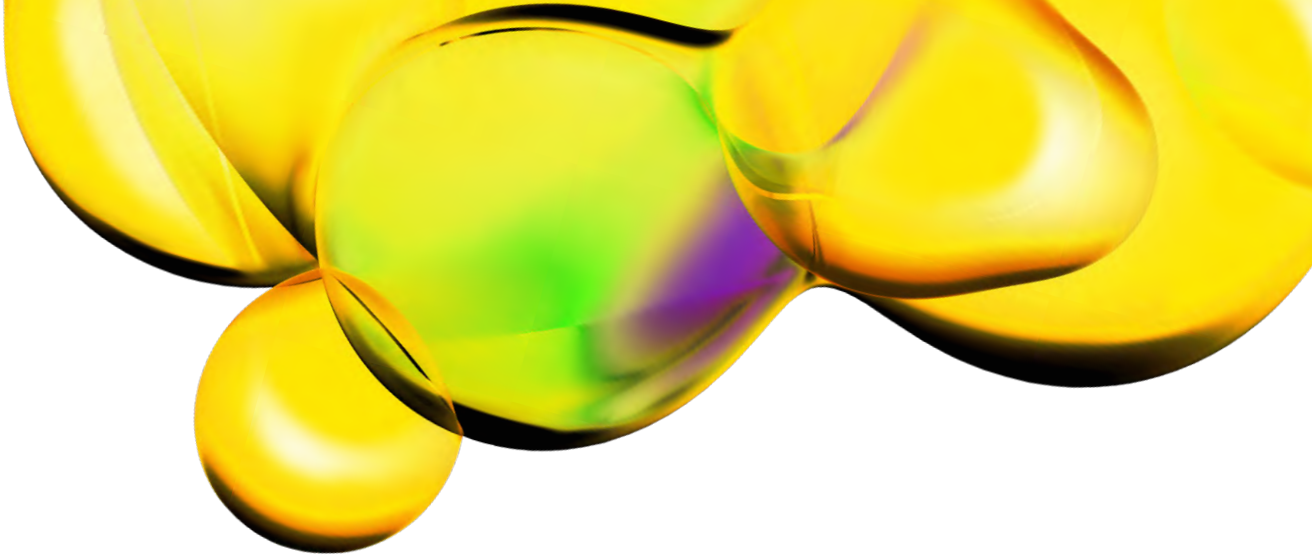
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example. Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting HTRF ratio versus log antibody concentration (M).

	[Standard] (nM)	Log [Standard] (M)	Ratio (1)	CV (2)
Negative control (Std 0)	0	-8.8	313	1.6%
Standard 1	2.9	-8.54	382	0.6%
Standard 2	4.9	-8.31	440	6.2%
Standard 3	8.4	-8.08	481	1.7%
Standard 4	14.3	-7.85	596	1.8%
Standard 5	24.2	-7.62	1131	0.8%
Standard 6	41.2	-7.39	1705	1%
Standard 7	70	-7.15	1600	0.9%





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