MANUAL

Technology: HTRF™ Biomarkers

HTRF Human IgG Detection Kit

Part number:	**6FHIGPEG**	**6FHIGPEH**
Assay points:	500 tests	10,000 tests

Storage: ≤ 60°C

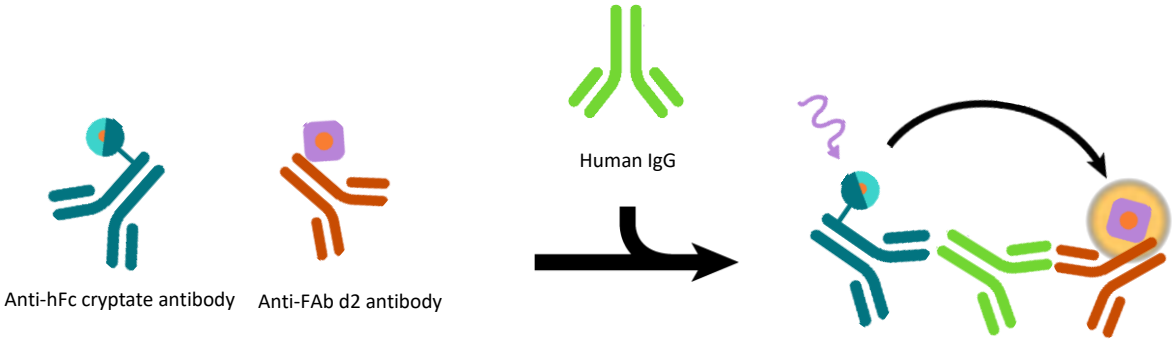
Version: 06 **Date:** June 2024

ASSAY DESCRIPTION

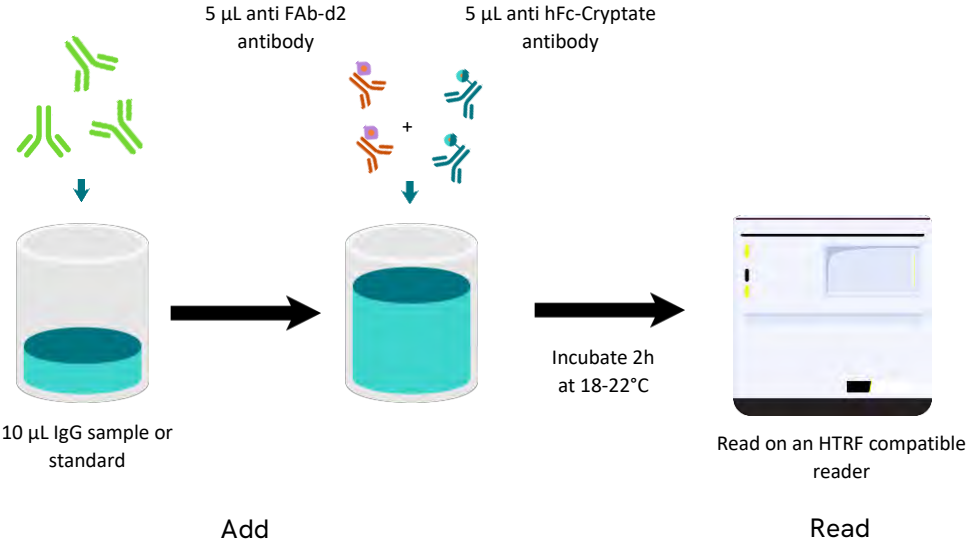
This assay is intended for the quantitative determination of human IgGs using the HTRF™ technology. The IgGs can be detected directly from cell supernatants or from purified solution.

As shown in the diagram to the right, IgGs are detected in a sandwich assay format using 2 different specific antibodies, one labeled 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). Reagents detection bind to the antibodies present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antibody-antibody complexes formed and therefore to the IgG concentration.



PROTOCOL AT A GLANCE



MATERIAL PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS		10 000 TESTS	
IgG Standard	≤ -20°C		Lyophilized		Lyophilized
Anti FAb-d2 antibody	≤ -20°C		50 µL/vial		1 mL/vial
Anti-hFc Cryptate-antibody	≤ -20°C		50 µL/vial		1 mL/vial
Diluent #1**	4°C to -20°C		20mL/vial		20mL/vial
Detection buffer #3	4°C to -20°C		7mL/vial		105mL/vial

*Diluent and detection buffer are shipped frozen, but can be stored at 2-8°C in your premises.
** Diluent #1 is available for separate purchase as a spare part #62DL1DDD

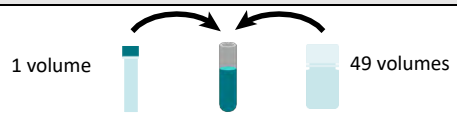
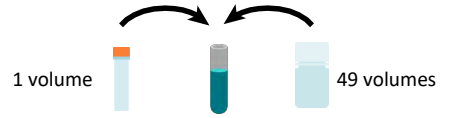
REAGENT PREPARATION

HTRF™ reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate- antibodies will impair the assay quality.
For an accurate quantitative determination of sample, dilution must be carried out with the medium used for preparing your samples.
Standard and antibodies may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of antibodies into disposable plastic vials for storage at –20°C or below.
Be careful, working solution preparation may differ between the 500 and the 10,000 data point kits.

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

To prepare working antibody solutions

Determine the amount of antibody needed for the experiment. Each well requires 5µL of each antibody.

500 TESTS	10 000 TESTS
Anti-Fab-d2 antibody	
	
Prepare a 50X diluted solution using the detection buffer #3: e.g. take 50 µL of d2-antibody stock solution and add it to 2450 µL of detection buffer #3.	Prepare a 50X diluted solution using the detection buffer #3: e.g. take 1000 µL of d2-antibody stock solution and add it to 49 mL of detection buffer #3.
Anti-hFc-Eu^{3+}-Cryptate-antibody	
	
Prepare a 50X diluted solution using the detection buffer #3: e.g. take 50 µL of cryptate antibody stock solution and add it to 2450 µL of detection buffer #3.	Prepare a 50X diluted solution using the detection buffer #3: e.g. take 1000 µL of cryptate antibody stock solution and add it to 49 mL of detection buffer #3

Standard curve preparation

Determine how many samples and replicates will be tested. It is recommended to use a reference Ab which can be considered as a standard. Determine how many standard levels and replicates will be tested. Each well requires 10µL of standard.

STANDARD	SERIAL DILUTIONS	WORKING CONCENTRATION (ng/mL)
Standard 8	150 µL standard stock solution + 150 µL diluent	2 000
Standard 7	100 µL standard 8 + 200 µL diluent	666.67
Standard 6	100 µL standard 7 + 200 µL diluent	222.22
Standard 5	100 µL standard 6 + 200 µL diluent	74.07
Standard 4	100 µL standard 5 + 200 µL diluent	24.69
Standard 3	100 µL standard 4 + 200 µL diluent	8.23
Standard 2	100 µL standard 3 + 200 µL diluent	2.74
Standard 1	100 µL standard 2 + 200 µL diluent	0.91
Standard 0	200 µL diluent	0

A recommended standard dilution procedure is listed and illustrated below.

→ Dilute the standard stock solution 2-fold with diluent; this yields the high standard (Std 8 : 2000 ng/mL) for the top of the curve. In practice:

- e.g. Reconstitute standard stock solution with the right volume indicated on the label. Take 150µL of standard stock solution and add it to 150µL of diluent. Mix gently.

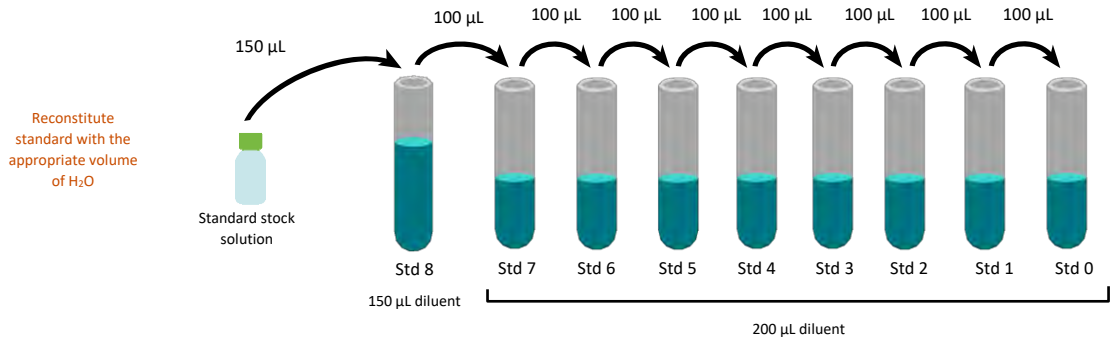
→ Use the high standard (Std 8) to prepare the standard curve using 1/3 serial dilutions as follows:

- Dispense 200µL of diluent in each vial from Std 7 to Std 1.
- Add 100µL of standard to 200µL of diluent, mix gently and repeat the 1/3 serial dilution to make standard solutions: 666.67, 222.22, 74.07, 24.69, 8.23, 2.74, 0.91 ng/mL. This will create 8 standards for the analyte.

Std 0 (Positive control) is diluent alone.

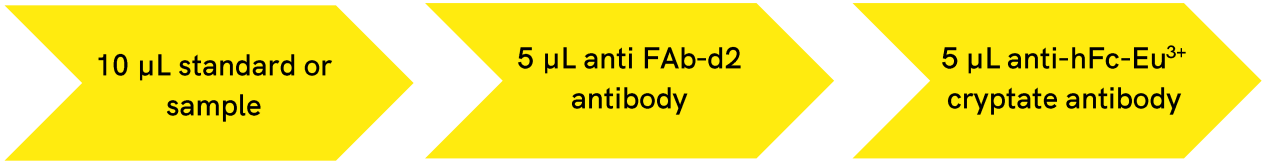
The standard dilution procedure is listed and illustrated below.

Dispense diluent in each vial.



ASSAY PROTOCOL

Dispense the reagents in the following order:



Please Note : It is possible to pre-mix the two antibodies just before dispensing and add 10µl of this mix.

→ Cover the plate with a plate sealer.
→ Incubate at 18-22°C for 2 hours.
→ Remove the plate sealer and,
→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF™ reader.

		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	-	-	-	10 µL
Diluent	10 µL	10 µL	10 µL	-
Anti-FAb-d2 antibody	5 µL	-	-	5 µL
Anti-hFc-Eu_3^+-Cryptate antibody	5 µL	5 µL	-	5 µL
Detection buffer #3	-	5 µL	10 µL	-

For more information about HTRF compatible readers, please visit our website.

DATA REDUCTION

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.

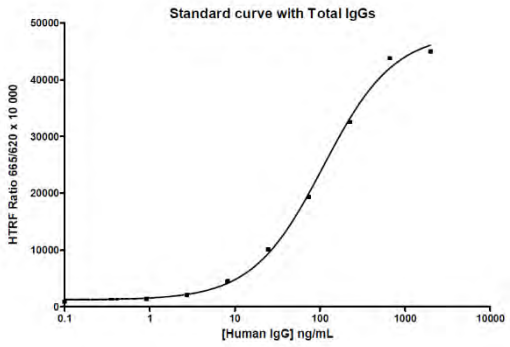
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit our website.

RESULTS

This data must not be substituted for that 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 drawn up by plotting Ratio 665/620 versus the analyte concentration:

Standard ng/mL		Ratio (1)	CV% (2)
Standard 0	Negative control	895	2.4%
Standard 1	0.91	1 395	8.4%
Standard 2	2.74	2 086	5.8%
Standard 3	8.23	4 497	4.7%
Standard 4	24.69	10 088	0.2%
Standard 5	74.07	19 366	2.3%
Standard 6	222.22	32 551	1.0%
Standard 7	666.67	43 832	1.2%
Standard 8	2 000	44 982	1.4%



To determine sample concentration, We recommend to use a log scale for the IgG concentrations and analyze the data with the sigmoidal dose response curve with variable slope.

REACH European regulations and compliance

This product and/or some of its components include a Triton concentration of 0.1% or more and as such, it is concerned by the REACH European regulations. We recommend researchers using this product to act in compliance with REACH and in particular: to only use the product for in vitro research in appropriate and controlled premises by qualified researchers, ii) to ensure the collection and the treatment of subsequent waste, and iii) to make sure that the total amount of Triton handled does not exceed 1 ton per year.

This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.

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

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