MANUAL

Technology: HTRF® Biomarkers

HTRF Human Fc kit

Part number	62HFCPEG	62HFCPEH
Test size	500 tests	10,000 tests

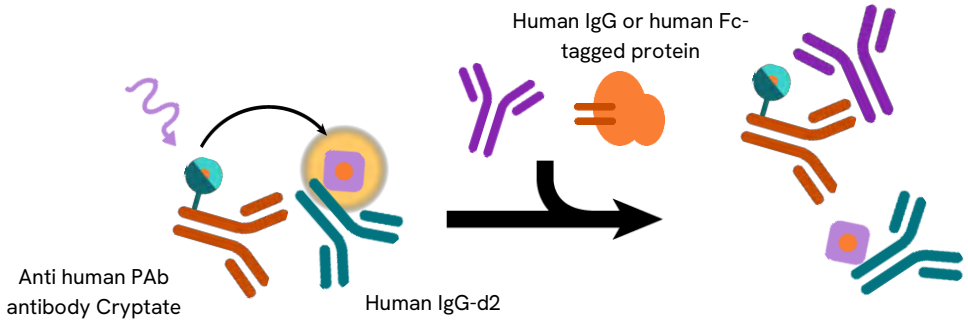
Storage: 2-8°C

Version: 06 **Date:** January 2024

ASSAY DESCRIPTION AND INTENDED USE

This kit is intended for the quantitative determination of human Fc-tagged proteins or human IgGs.

The principle of this competitive immunoassay is based on HTRF® technology. As shown beside, human IgG (hIgG) or hFc-tagged proteins can displace the binding between IgG labeled with d2 and PAb anti-human Fc labeled with Europium Cryptate.
Specific signal (i.e. energy transfer) is inversely proportional to the concentration of human Fc in the sample or standard.



BACKGROUND

The production of human-Fc tagged chimera or of humanized monoclonal antibodies has raised considerable interest as potential drug candidates, but the screening of these libraries may be slowed when using conventional methodologies.

This kit enables human-Fc chimera from various origins as well as all human IgGs subclasses to be detected and quantified within 3 hours.

PROTOCOL

Supplied reagents

Supplied reagents	500 TESTS		10,000 TESTS	
	Reagent reconstitution (stock solutions)	**Working solutions**	**Reagent reconstitution (stock solutions)**	**Working solutions**
Human Fc Eu-Cryptate antibody 1 vial, lyophilized	Add 2.5 mL of detection buffer to each vial. Mix gently.	Ready-to-use after reconstitution	Reconstitute each vial with 2.5 mL of distilled water. Mix gently.	For each vial dilute 1 volume of reconstituted reagent in 19 volumes of detection buffer (e.g. for 10,000 tests: 2.5 mL of reconstituted reagent + 47.5 mL of detection buffer). Mix gently.
Human IgG-d2 reagent 1 vial, lyophilized				
Human IgG standard. Concentrated human IgGs. 1 vial, lyophilized	See label indications for reconstitution volume. Mix gently after reconstitution	See standard curve preparation for further dilution	See label indications for reconstitution volume. Mix gently after reconstitution	See standard curve preparation for further dilution
Detection buffer 1 vial - 7 mL (500 tests) #2 1 vial - 105 mL (10,000 tests) #3		Ready-to-use Detection buffer2		Ready-to-use Detection buffer 3
Diluent 1 vial - 20 mL		Ready-to-use		Ready-to-use

Detection reagent working solutions must be prepared in distinct vials and dispensed separately.

Allow the reagents to warm up at room temperature for at least 30 minutes and reconstitute all vials as indicated above.

Precaution: HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the detection reagents: IgG-d2 reagent and Eu-Cryptate antibody will impair the assay's quality.

Reagent stability

All reagents should be stored at 2-8°C until reconstituted. Under proper storing conditions, they are stable until the expiry date indicated on the labels.

Reconstituted reagents (stock and working solutions) are stable for up to one week at 4°C. They can be refrozen (at -60°C or below) and thawed one more time.

Standard curve preparation

Follow the dilution sequence shown in the table below to constitute the standard curve. Dilution must be carried out with the diluent or appropriate medium according to samples.

Standard	Preparation	hIgG concentration in ng/mL
Standard 7	Reconstituted reagent (pure)	4 000
Standard 6	100 µL Std 7 + 200 µL diluent	1 333
Standard 5	100 µL Std 6 + 200 µL diluent	444
Standard 4	100 µL Std 5 + 200 µL diluent	148
Standard 3	100 µL Std 4 + 200 µL diluent	49
Standard 2	100 µL Std 3 + 200 µL diluent	16.5
Standard 1	100 µL Std 2 + 200 µL diluent	5.5

* [hIgG] is indicated on the label of the standard. It corresponds to the concentration of the solution obtained after reconstitution with distilled water.

Sample preparation

Dilute all samples to be assayed with the diluent or with freshly made PO_4 50 mM, BSA 0.2% pH 7 buffer. Consecutive dilutions should be made within the 5,5 to 4 000 ng/mL (37 pM to 27 nM) range (working solution).

Assay protocol for 96 & 384-well white low volume plates

→ Dispense the reagents in the following order:

- 10 µL standard or sample*
- 5 µL Human-IgG-d2 reagent
- 5 µL Human Fc Eu Cryptate antibody

* For negative control, replace human-IgG-d2 by 5 µL of detection buffer and standard by 10 µL of diluent.

* For positive control, replace standard by 10 µL of diluent.

→ Cover the plate with a plate sealer and leave to incubate at room temperature from 2 h 30 to over night.

→ Read on a compatible HTRF® reader

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website.

Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 500 tests using a 384- well low volume plate in 20 µL final assay volume.

To move to other plate formats (96 half-well or 1536-well) and final volumes (100 µL to less than 10 µL), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 µL final assay volume. For instance, in the case of the 1536-well format in 10 µL final volume, half as much material per well is used, thereby allowing 1,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

		Assay format		
Assay components	**Volume proportion**	**1536-well (10 µL)**	**384-well low volume (20µL)**	**96 half-well (100 µL)**
Sample	2 volumes	5 µL	10 µL	50 µL
Human-IgG-d2	1 volume	2.5 µL	5 µL	25 µL
Anti-Human Fc Cryptate antibody	1 volume	2.5 µL	5 µL	25 µL
62HFCPEG		1,000 tests	500 tests	100 tests
62HFCPEH		20,000 tests	10,000 tests	2,000 tests

Data reduction

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

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

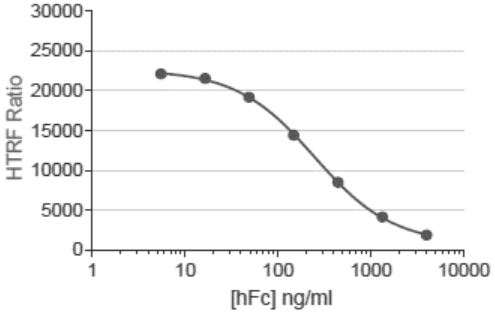
2) Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

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

An example of data reduction is given in the table below (readout on PHERAstar Plus). These data should not be substituted for results obtained in the laboratory.

Draw up the standard curve by plotting Ratio versus hIgG concentration as shown in the graph below.

	Ratio	CV%
Negative control	376	1.0%
Std 0 – 0 ng/mL	22 544	1.0%
Std 1 – 5.5 ng/mL	22 144	1.0%
Std 2 – 16.5 ng/mL	21 551	0.0%
Std 3 – 49 ng/mL	19 218	1.0%
Std 4 - 148 ng/mL	14 440	3.0%
Std 5 – 444 ng/mL	8 520	2.0%
Std 6 – 1 333 ng/mL	4 156	1.0%
Std 7 – 4 000 ng/mL	1 885	0.0%



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

Assay characteristics

The table summarizes the characteristics of the assay relative to the detection limit (hIgG concentration corresponding to the “dose of mean zero- 2SD”) and the EC50 (hIgG concentration which allows the displacement of 50% of binding). This data has been obtained using the reference PHERAstar FS reader (BMG LABTECH).

	Detection limit	EC50
2 h 30 to over night at room temperature	≤ 9 ng/mL	216 - 296 ng/mL

To obtain additional information or support, please contact the HTRF technical support team.

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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