R&D, Administration and Europe Office
Cisbio Bioassays
Phone: +33 (0)4 66 79 67 05
Fax: +33 (0)4 66 79 19 20
E-mail: bioassays@cisbio.com

Japan Office
Sceti Medical Labo K.K.
Phone: +81 (0)3 5510 2932
Fax: +81 (0)3 5510 0130
E-mail: reagent@scetimedilabo.co.jp

USA Office
Cisbio US, Inc.
Phone : 888 963 4567
Fax : 781 687 1500
E-mail : htrfinfo@cisbio.us

htrf

www.htrf.com

HTRF® package insert

Document reference : 62HFCPEC rev08 (Oct. 2010)

Human total IgG kit
20,000 tests

For in vitro research use only
Storage temperature : 2-8°C

Packaging details :

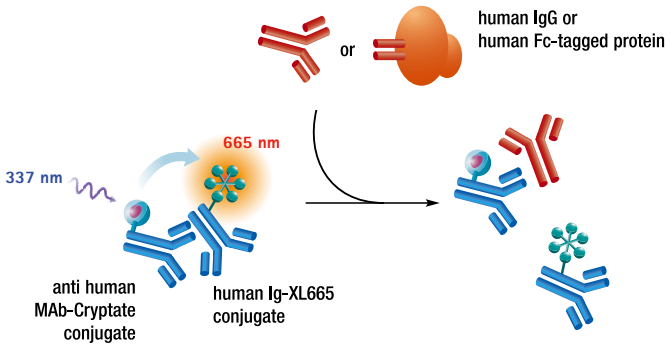
	384-well low volume plate (20 µL)
62HFCPEC	20,000 tests

1. Assay description and intended use

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

The principle of this competitive immunoassay is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown below, human IgG (hIgG) or hF_c-tagged proteins can displace the binding between IgG labeled with XL665 and Pab anti-human F_c labeled with Cryptate.

Specific signal (i.e. energy transfer) is inversely proportional to the concentration of human F_c in the sample or standard.



2. Background

The production of human-F_c 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.

3. Protocol

3.1. Supplied reagents

Supplied reagents	Reagent reconstitution (stock solutions) *		Working solutions
Anti-human-IgG F_c-Cryptate** 1 vial, lyophilized	Reconstitute each vial with 5 mL of distilled water. Mix gently.	⇨	For each vial dilute 1 volume of reconstituted reagent in 19 volumes of reconstitution buffer (e.g. for 10,000 tests: 2.5 mL of reconstituted reagent + 47.5 mL of reconstitution buffer). Mix gently.
Human-IgG-XL665 1 vial, lyophilized			
Human total IgG calibrator. Concentrated human IgGs. 1 vial, lyophilized	See label indications for reconstitution volume. Mix gently after reconstitution	⇨	See calibration curve preparation for further dilution

Supplied reagents (continued)	**Reagent reconstitution (stock solutions)***		**Working solutions**
Human total IgG control. Free human IgG assay control. 1 vial, lyophilized	See label indications for reconstitution volume. Mix gently after reconstitution	⇨	To be used directly after reconstitution

Reconstitution buffer (200 mL) 50 mM Phosphate buffer, pH 7.0, 0.8M KF, 0.2 % BSA
Diluent (20 mL) 50 mM Phosphate buffer, pH 7.0, 0.2 % BSA, 0.02 % NaN_3, preservatives

Note : Supplementary human-IgG maximum calibrator (ref 62HFCCDA), human-IgG assay control (ref 62HFCTDA) and diluent (ref 62DL1DDD) can be obtained separately on request.

* All lyophilized reagents must be reconstituted with distilled water.

** The Cryptate conjugate concentration was optimized in order to ensure an average counting of 40,000 cps at 620 nm (384-well low volume format), using the reference PHERAstar Plus reader (BMG LABTECH).

Conjugate 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 XL665 and Cryptate-conjugates will impair the assay's quality.

3.2. 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 -80°C) and thawed one more time.

3.3. Calibration curve preparation

Follow the dilution sequence shown in the table below to constitute the calibration curve. Dilution must be carried out with the diluent or with freshly made PO_4 50 mM, BSA 0.2% pH7.

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

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

3.4. Control

Once reconstituted, each vial of IgG control enables at least 45 replicates in 384-well low volume format. Unused control solution should be divided into aliquots and stored at -80°C. Refer to label indications for IgG concentration after reconstitution.

3.5. 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 4000 ng/mL (37 pM to 26 nM) range (working solution).

3.6. Assay protocol for 384-well low volume plate

⇨ Dispense the reagents in the following order :

- 10 µL standard or sample*
- 5 µL Human-IgG-XL665
- 5 µL Anti-human-IgG F_c-Cryptate

* For negative control, replace human-IgG-XL665 by 5 µL of reconstitution 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 1/2 hours to over night.

⇨ Read on a compatible HTRF® reader (more information about compatible reader at htrf-assays.com/reader)

3.7. Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 20,000 tests using a 384- well low volume plate in 20 µL final assay volume (HTRF® packaged basis).

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 40,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

Assay components	Volume proportion	Assay format		
		1536-well (10 µL)	384-well low volume (20 µL)	96 half-well (100 µL)
Sample	2 volumes	5 µL	10 µL	50 µL
XL665 conjugate	1 volume	2.5 µL	5 µL	25 µL
Cryptate conjugate	1 volume	2.5 µL	5 µL	25 µL
	Bulk size	40,000 tests	20,000 tests	4,000 tests

Plate references : 96 half-well plate (Costar # 3694 or equivalent), 384-well low volume plate (Greiner # 784076), 1536-well (Greiner # 782086)

3.8. Data reduction

Results are calculated from the 665nm / 620nm ratio and expressed in Delta F. 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 calibration curve by plotting delta F% versus hIgG concentration as shown in the graph on the right.

	A (665nm)	B (620nm)	Ratio (1)	Mean Ratio (2)	CV % (3)	Delta F % (4)
Negative control	2,042 2,226	40,574 44,037	503 505	504	0.3 %	0
[calibrator] ng/mL initial						
0 Positive control	23,312 22,811	42,443 42,305	5,493 5,392	5,442	1.3 %	979
5.5	22,251 22,314	41,302 41,992	5,387 5,314	5,351	1.0 %	961
16.5	21,517 21,714	42,068 42,277	5,115 5,136	5,125	0.3 %	716
49	18,993 18,755	42,081 41,371	4,513 4,533	4,523	0.3 %	797
148	13,857 13,658	42,174 40,526	3,286 3,370	3,286	1.8 %	551
444	9,209 9,229	43,090 42,983	2,137 2,147	2,142	0.3 %	235
1,333	6,081 6,341	44,292 44,915	1,373 1,412	1,392	2.0 %	176
4,000	4178 4180	43126 44967	969 930	949	2.8 %	88
Human IgG control	13,561 14,039	44,375 44,679	3,056 3,142	3,099	2.0 %	1,202

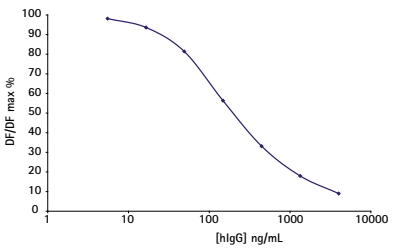
1. $\text{Ratio} = \frac{A_{665nm}}{B_{620nm}} \times 10^4$

2. $\text{Mean Ratio} = \frac{\Sigma \text{ratios}}{2}$

3. $\text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$

4. $\text{Delta F} = \frac{\text{Calibrator or sample Ratio} - \text{Ratio}_{neg}}{\text{Ratio}_{neg}} \times 100$

(Ratio_{neg} = negative control)



The human total IgG control validates the accuracy of the calibration curve. The concentration deduced from the delta F obtained should fall into the concentration range indicated on the label of the vial.

3.9. 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 EC_{50} (hIgG concentration which allows the displacement of 50% of binding). This data has been obtained using the reference PHERAstar Plus reader (BMG LABTECH).

	Detection limit	EC_{50}
2 1/2 h (or O. N.) at room temperature	≤ 9 ng/mL	226 ng/mL

cisbio Bioassays
Member of IBA group