Headquarters & Europe Office
Cisbio Bioassays
Phone: +33 (0)4 66 79 67 05
Fax: +33 (0)4 66 79 19 20
bioassays@cisbio.com

USA Office
Cisbio US, Inc.
Phone: +1 888 963 4567
Fax: +1 781 687 1500
htrfinfo@cisbio.us

China Office
Cisbio China
Phone: +86 21 5018 9880
Fax: +86 21 5020 3055
htrfinfoasia@cisbio.cn

Japan Office
Cisbio Japan
Phone: +81-(0)43-306-8712
Fax: +81-(0)43-306-8713
htrf@cisbio.jp

www.cisbio.com

Product information

Document reference : 6FR3APAG/E/F (Rev 02 / Nov. 2020)

CD16 (F158)Cellular Binding Assay

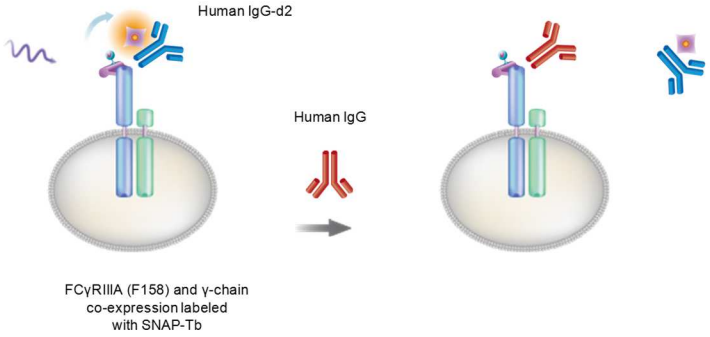
For in vitro research use only
Reagent storage temperature: -60°C or below

Packaging details :

	384-well low volume plate (20 µl)
6FR3APAG	100 tests
6FR3APAE	5 x 100 tests
6FR3APAF	10 x 100 tests

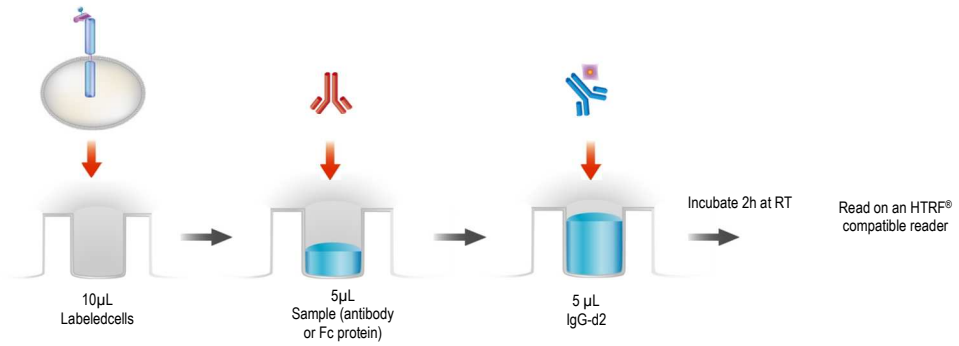
1. Assay description

This assay was developed to accurately and efficiently measure the binding of different subclasses of IgG on the CD16 (FcγRIII) (F158)receptor using the Tag-lite® technology.
As shown on the diagram below, the assay uses HEK293 cells expressing the CD16 receptor labeled with Cisbio's Terbium donor dye. In the assay, unlabeled antibodies compete with an acceptor labeled human IgG (IgG-d2) for binding to the receptor.



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).
The unlabeled Ab present in the sample, competes for binding on the CD16 receptor with the IgG-d2 conjugate and thereby prevents FRET from occurring. The specific signal modulates negatively and is inversely proportional to IgG affinity for CD16 receptor.

2. Protocol at a glance



3. HTRF® reagents

	IgG-d2 conjugate	Prelabeled cells with CD16 (F158)/γ chain	Tag-lite buffer (5X)
	 purple cap	 clear cap	 yellow cap
Stock solution	/	/	1.5mL/vial
Storage	-20°C or below	-80°C or below	-4°C to -20°C*

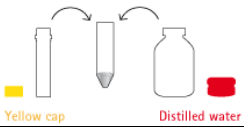
Be careful: the IgG-d2 is strictly restricted to use of this particular receptor and can not be applied to another one.

4. Reagentpreparation

Thaw the IgG-d2 and Tag-lite buffer at room temperature.
Prepare the working solutions from stock solutions (§3) by following the instructions below. The cells need to be prepared after all of the reagents are ready.

4.1. Preparation of conjugate workingsolutions

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

Tag-lite buffer 5X	IgG-d2 conjugate
1 volume 4 volumes 	Add 540µL 
Prepare a 1X Tag-lite buffer: e.g. add 6 mLof distilled water to the 1.5 mL stock solution.	Add 540µl of Tag-lite buffer to the IgG-d2 stock solution.

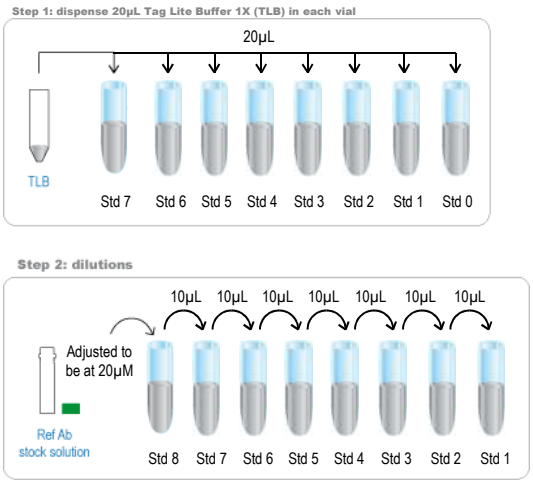
4.2. Standard curve preparation

Determine how many samples and replicates to be tested. Each well requires 5µL of sample.
It is recommended to use a reference Ab which can be considered as a standard.
To determine the EC50 of the IgGs, it is recommended that you test a wide range of unlabeled IgG concentrations. All IgGs tested should be diluted in Tag-lite buffer (1X) (for example up to 5 or 20 µM (3mg/mL) with 3 fold dilution).

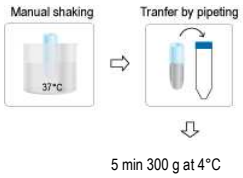
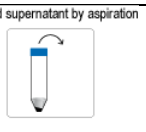
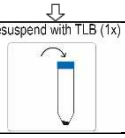
Due to the difference of affinity, one standard IgG dilution cannot be applied to every FcR. The IgG concentration needs to be adapted for each variant tested.

Standards	Working concentration (nM)	Preparation
Std 8	20000	See indications below
Std 7	6666.6	10µLStd 8 + 20µLTag-lite buffer
Std6	2222.2	10µLStd 7 + 20µLTag-lite buffer
Std5	740	10µLStd 6 + 20µLTag-lite buffer
Std4	247	10µLStd 5 + 20µLTag-lite buffer
Std3	82.3	10µLStd 4 + 20µLTag-lite buffer
Std2	27.4	10µLStd 3 + 20µLTag-lite buffer
Std1	9.1	10µLStd 2 + 20µLTag-lite buffer
Std0	0	30µLTag-lite buffer

The standard dilution procedure is listed and illustrated below.



4.3. Cells preparation

1	Prepare a conical vial (A) (ex: Falcon 50 mL) containing 15 mL of PBS at 4°C.	
2	Thaw labeled frozen cells at 37°C (water bath, manual shaking) until all the ice is thawed (1-2 min) and transfer them quickly by pipetting into the vial prepared above.	
3	Centrifuge 5 min at 300 g at 4°C.	
4	Gently remove supernatant by aspiration. Be careful the pellet may not be visible.	
5	Resuspend the pellet in 1.1 mL of Tag-lite Buffer (1X) with a P1000 pipette.	
6	Dispense 10µL per well of this cell suspension in a 384 small volume plate.	

Recommendations:

- HTRF® reagent concentrations have been calibrated for optimal assay performance. Note that any dilution or improper use of the d2 conjugate or pre-labeled cells will impair the quality of the assay.
- For an accurate quantitative determination of sample, dilution must be carried out in Tag-lite buffer.
- The labeled cells need to be resuspended regularly before dispensing to keep an homogeneous number of cells/well.

To obtain additional information or support, please contact your technical support team (htrfservices@cisbio.com).

5. Assay protocol

Dispense the reagents in the following order:

10µL Pre-labeled cells → 5µL Human IgG → 5µL IgG-d2 conjugate

DO NOT pre-mix the labeled cells and the IgG-d2 conjugate.

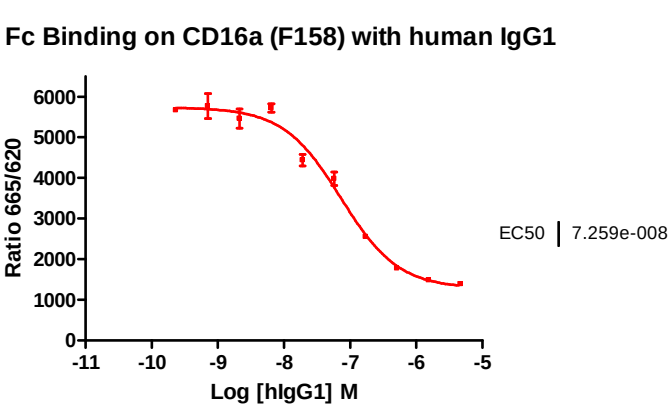
→ Cover the plate with a plate sealer.
→ Let theincubation take place at room temperature for 2 hours.
→ Remove the plate sealer.
→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on anHTRF® compatible reader.
(more information about compatible reader at www.cisbio.com/compatible-readers/)

	Assay controls			
	Negative control	Maximum FRET	Buffer control	Sample / Std
	Used to check the Cryptate signal at 620nm	*to know the maximalsignal without competition*	*used to check background fluorescence*	
Pre-labeled cells	10 µL	10 µL	-	10 µL
Tag-lite buffer	10 µL	5 µL	20 µL	-
Sample / Std	-	-	-	5 µL
IgG-d2 conjugate	-	5 µL	-	5 µL

6. Data reduction

This data must not be substituted for that obtained in the laboratory and should be considered only as an example (readouts on PHERAstar[FS]). Results may vary from one HTRF® compatible reader to another.

Standard nM (final concentration)	Mean Ratio 665/620 (1)	CV % (2)
Std 1– 0.24	5662	0.5
Std 2–0.71	5769	1.0
Std 3–2.13	5460	7.6
Std 4–6.4	5721	6.2
Std 5– 19.2	4436	2.6
Std 6– 57.6	3980	4.6
Std 7– 172.8	2549	5.8
Std 8 - 518.5	1771	2.2
Std 9 - 1555.6	1476	4.6
Std 10 - 4666.7	1387	4.2



Ratio (1)	$\frac{\text{Signal}_{665nm}}{\text{Signal}_{620nm}} \times 10^4$	Ratio must be calculated for each individual well.
CV% (2)	$\frac{\text{Standard deviation}}{\text{Mean ratio}} \times 100$	The mean and standard deviation can then be worked out from ratio replicates.
For more information about data reduction, please visit our website at: www.cisbio.com/htrf-ratio-and-data-reduction/		