

MANUAL

Technology: HTRF®

HTRF CD16a (FcγRIIIa, V158) Cellular Binding Kit

Part number	62C16PAG	62C16PAE	62C16PAF
Test size	100 tests	5 x 100 tests	10 x 100 tests

Storage: ≤-60°C

Version: 10

Date: December 2023

ASSAY PRINCIPLE

This assay was developed to accurately and efficiently measure the binding of different subclasses of IgG on the CD16a receptor using the Tag-lite® technology. The kit may also be used to study how antibody fucosylation differentially impacts binding affinity.

As shown on the diagram below, the assay uses HEK293 cells expressing the CD16a receptor co-expressed with the functionally relevant Gamma chain labeled with Revvity's Terbium donor dye. In the assay, unlabeled antibodies compete with an acceptor labeled human IgG (IgG-d2) for binding to the receptor.

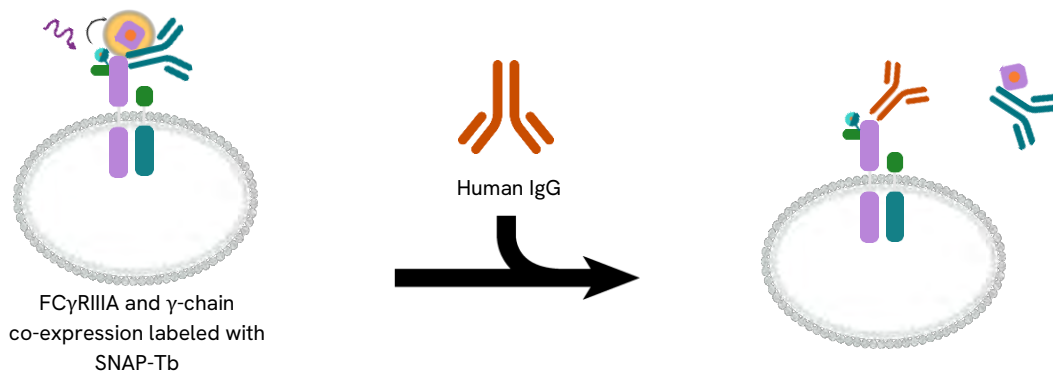
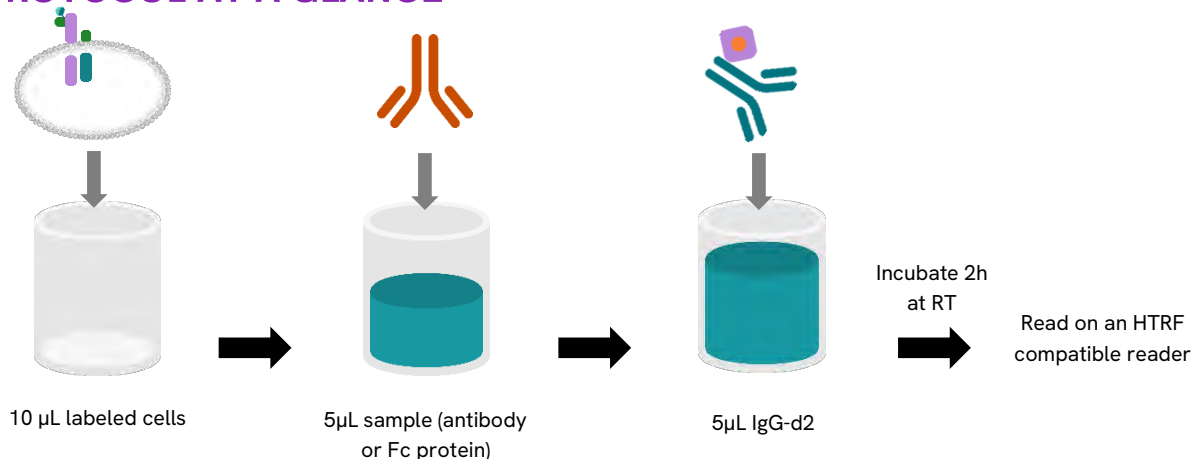


Figure 1: Principle of the assay.




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 CD16a receptor with the IgG-d2 reagent and thereby prevents FRET from occurring. The specific signal modulates negatively and is inversely proportional to IgG affinity for CD16a receptor.

PROTOCOL AT A GLANCE



MATERIAL PROVIDED

Reagents			Stock solution	Storage
IgG-d2 reagent		purple cap	/	-16°C or below
Prelabeled cells with CD16a		transparent cap	/	-60°C or below
Tag-lite buffer (5X)		Yellow cap	1.5mL/vial	2-8°C

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

Purchase separately

96-well or 384-well small volume (SV) detection microplates - For more information about microplate recommendations, please visit our website.

For HTRF certified reader

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

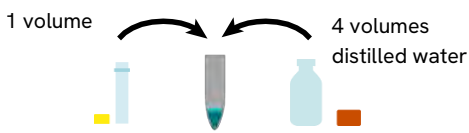

REAGENT PREPARATION

Thaw the IgG-d2 and Tag-lite buffer at room temperature.

Prepare the working solutions from stock solutions by following the instructions below. The cells need to be prepared after all of the reagents are ready.

Preparation of conjugate working solutions

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

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

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.

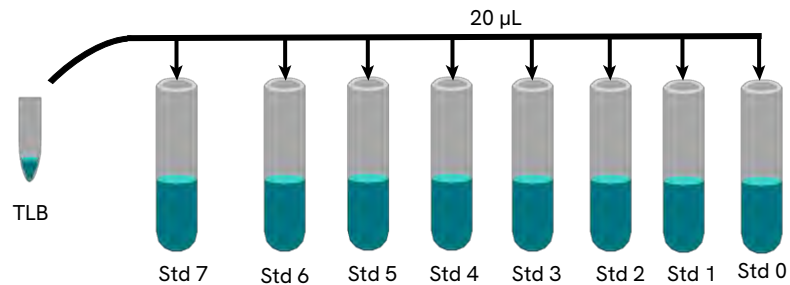
To determine the EC_{50} of the IgGs, it is recommended that you test a wide range of unlabeled IgG concentrations. All IgGs tested should be diluted in Tag-lite buffer (1X) (for example up to 10 or 20 µM with 3-fold dilution).

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.

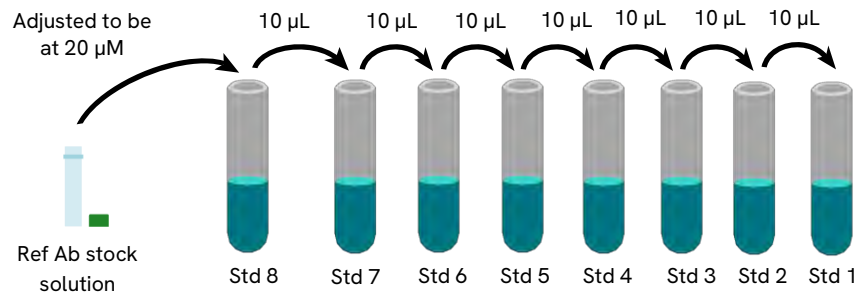
STANDARD	PREPARATION	WORKING CONCENTRATION (nM)
Standard 8	Reference Ab to dilute with Tag-lite buffer	20 000
Standard 7	10 µL Std 8 + 20 µL Tag-lite buffer	6 666.7
Standard 6	10 µL Std 7 + 20 µL Tag-lite buffer	2 222.2
Standard 5	10 µL Std 6 + 20 µL Tag-lite buffer	740.7
Standard 4	10 µL Std 5 + 20 µL Tag-lite buffer	246.9
Standard 3	10 µL Std 4 + 20 µL Tag-lite buffer	82.3
Standard 2	10 µL Std 3 + 20 µL Tag-lite buffer	27.4
Standard 1	10 µL Std 2 + 20 µL Tag-lite buffer	9.1
Standard 0	30 µL Tag-lite buffer	0

The standard dilution procedure is listed and illustrated below.


Step 1: dispense 20 µL Tag Lite Buffer (1X) (TLB) in each vial.



Step 2: dilutions



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.	Manual shaking → Transfer by pipeting
3	Centrifuge 5 min at 300 g at 4°C	
4	Gently remove supernatant by aspiration. Be careful the pellet may not be visible.	Discard supernatant by aspiration
5	Resuspend the pellet in 1.1 mL of Tag-lite Buffer (1X) with a P1000 pipette	Resuspend with TLB (1X)
6	Dispense 10µL per well of this cell suspension in a 384 small volume plate	

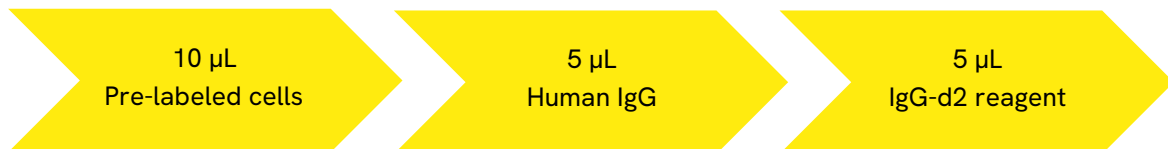
Recommendations:

- 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.
- IgG-d2 reagent may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of conjugate into disposable plastic vials for storage at $\leq -16^{\circ}\text{C}$.
- 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.

ASSAY PROTOCOL

Dispense the reagents in the following order:



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

- Cover the plate with a plate sealer.
- Let the incubation take place at room temperature for 2 hours.
- Remove the plate sealer.
- Read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

	Assays controls			
	Negative control	Maximum FRET	Buffer control	Sample / Std
	<i>used to check the Cryptate signal at 620nm</i>	<i>to know the maximal signal without competition</i>	<i>used to check background fluorescence</i>	
Pre-labeled cells	10 µL	10 µL	-	10 µL
Tag-lite buffer	10 µL	5 µL	20 µL	-
Sample / Std	-	-	-	5 µL
IgG-d2 reagent	-	5 µL	-	5 µL

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.

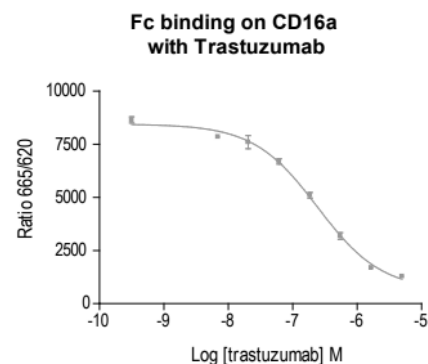
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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

RESULTS

These 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.

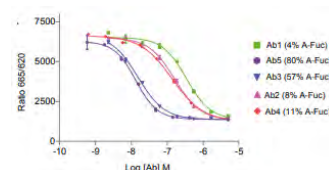
Standard	Working	Final	Mean Ratio 665/620 (1)	CV % (2)
	Concentration (nM)			
Standard 1	9.1	0.3	8 250	0.13
Standard 2	27.4	6.9	7 361	0.03
Standard 3	82.3	20.6	7 262	1.49
Standard 4	246.9	61.7	6 416	1.29
Standard 5	740.7	185.2	4 704	0.17
Standard 6	2 222.2	555.6	2 895	4.46
Standard 7	6 666.7	1 666.7	1 643	1.18
Standard 8	20 000	5 000	1 238	0.86



How to compare the fucosylation degree on antibodies

A known antibody with 50% fucosylation for example can be used as a reference to compare with the other antibodies to test. Cf graph shown.

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.



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.



The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF

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

www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2023, Revvity, Inc. All rights reserved.