MANUAL

Technology: HTRF®

HTRF CD32 (FcγRIIB) Cellular Binding Kit

Part number	6FR2BPAG	6FR2BPAE	6FR2BPAF
Test size	100 tests	5 x 100 tests	10 x 100 tests

Storage: ≤-60°C

Version: 03 **Date:** January 2024

ASSAY PRINCIPLE

This assay was developed to accurately and efficiently measure the binding of different subclasses of IgG on the FcgRIIB receptor using the Tag-lite® technology.

As shown on the diagram below, the assay uses HEK293 cells expressing the FcgRIIB receptor 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.

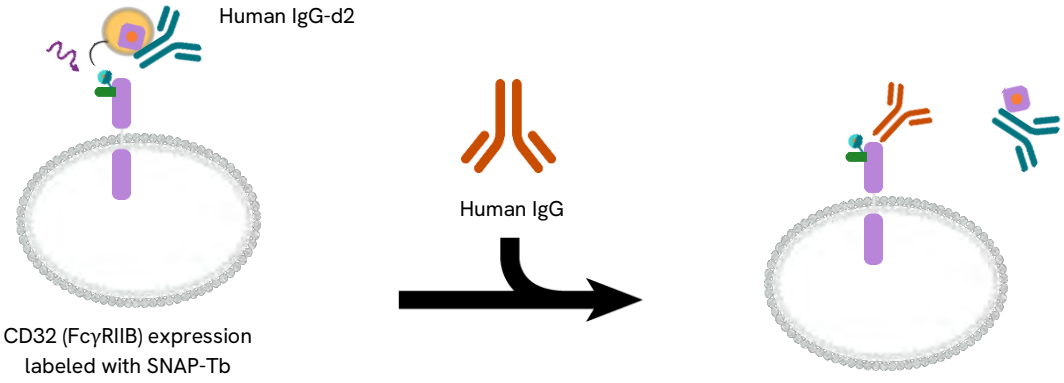
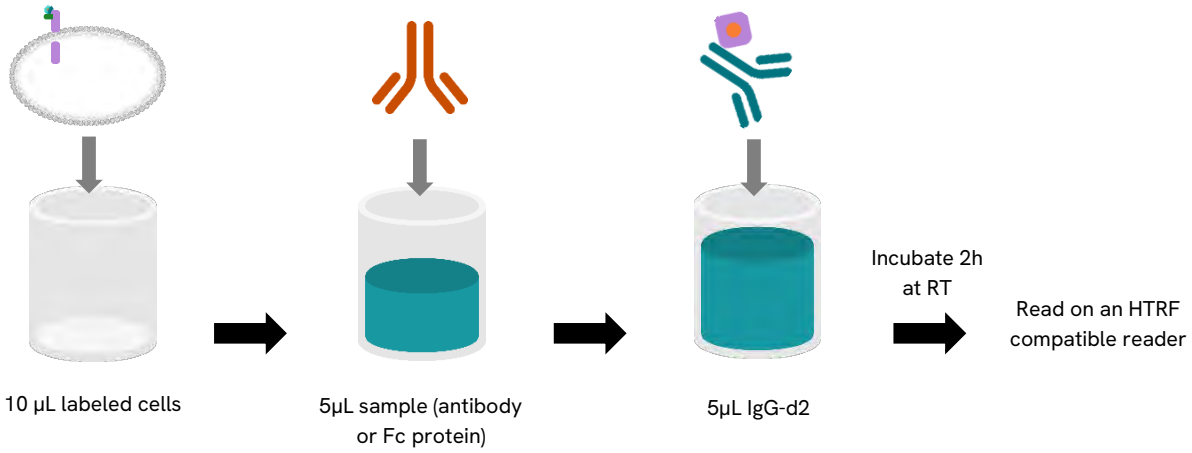


Figure 1: Principle of the assay.

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 FcgRIIB 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 FcgRIIB receptor.

PROTOCOL AT A GLANCE



MATERIAL PROVIDED

Reagents			Stock solution	Storage
IgG-d2 conjugate		purple cap	/	-20°C or below
Prelabeled cells with FcγRIIB		transparent cap	/	-60°C or below
Tag-lite buffer (5X)		Yellow cap	1.5mL/vial	-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.

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

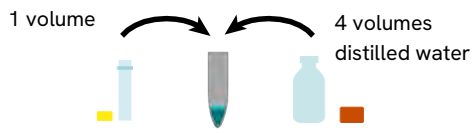
For more information about HTRF® compatible readers and for set-up recommendations, please visit our website.

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 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.

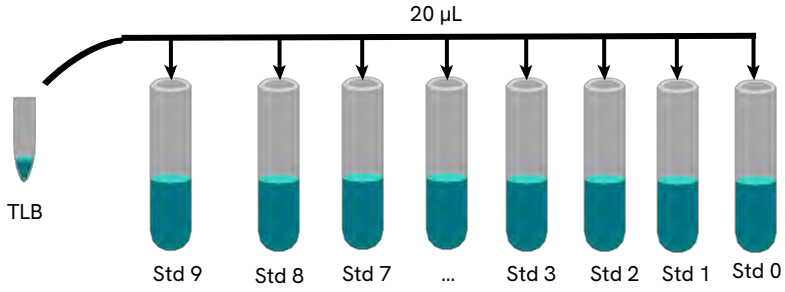
To determine the EC_{50} of the IgGs, it is recommended that you test a wide range of unlabeled IgG concentrations. All IgGs tested should be diluted in Tag-lite buffer (1X) (for example up to 10 or 20 µM with 3-fold dilution).

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.

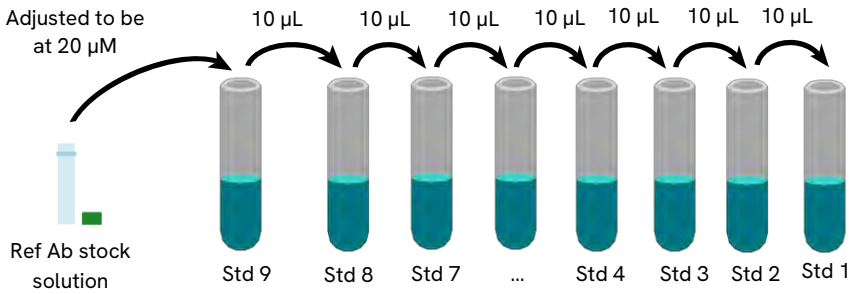
STANDARD	PREPARATION	WORKING CONCENTRATION (nM)
Standard 10	See indications below	20 000
Standard 9	10 µL Std 10 + 20 µL Tag-lite buffer	6 666.6
Standard 8	10 µL Std 9 + 20 µL Tag-lite buffer	2 222.2
Standard 7	10 µL Std 8 + 20 µL Tag-lite buffer	740
Standard 6	10 µL Std 7 + 20µL Tag-lite buffer	247
Standard 5	10 µL Std 6 + 20 µL Tag-lite buffer	82.3
Standard 4	10 µL Std 5 + 20 µL Tag-lite buffer	27.4
Standard 3	10 µL Std 4 + 20 µL Tag-lite buffer	9.1
Standard 2	10 µL Std 3 + 20 µL Tag-lite buffer	3.0
Standard 1	10 µL Std 2 + 20 µL Tag-lite buffer	1.0
Standard 0	30 µL Tag-lite buffer	0

The standard dilution procedure is listed and illustrated below.

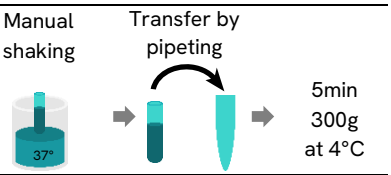
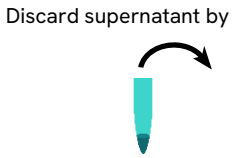
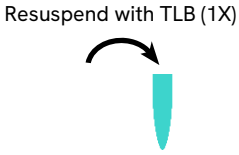
Step 1: dispense 20 µL Tag Lite Buffer (1X) (TLB) in each vial.



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.	
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	

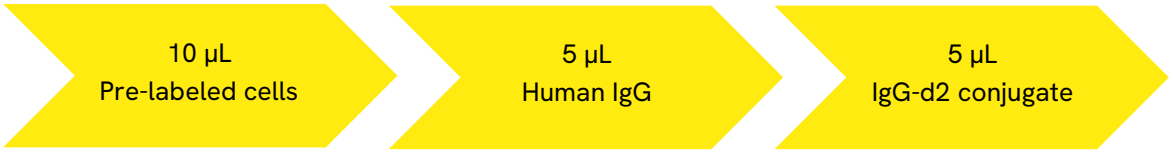
Recommendations:

- 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.

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
	used to check the Cryptate signal at 620nm	*to know the maximal signal 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

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.

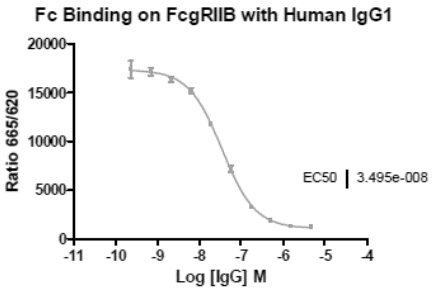
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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 nM	Working final concentration (nM)	Final final concentration (nM)	Mean Ratio 665/620 (1)	CV % (2)
Standard 1	1.0	0.03	17 376	7.1
Standard 2	3.0	0.1	17 143	3.5
Standard 3	9.1	0.3	16 333	2.4
Standard 4	27.4	6.9	15 194	2.7
Standard 5	82.3	20.6	11 728	1.9
Standard 6	247	61.7	7 205	6.3
Standard 7	740	185.2	3 298	2.4
Standard 8	2 222.2	55.6	1 891	7.4
Standard 9	6 666.6	1 666.7	1 320	3.1
Standard 10	20 000	5 000	1 265	0.4



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.

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