MANUAL

Technology: HTRF™ Biomarkers

HTRF FcRn Binding Kit

Part number	64FCRNPET	64FCRNPEG	64FCRNPEH
Test size	100 tests	500 tests	10,000 tests

Storage: ≤-16°C or below

Assay volume: 20 µL

Version: 08 **Date:** July 2025

ASSAY PRINCIPLE

This kit is intended for monitoring the binding of the IgG Fc region to the human FcRn receptor in buffered solution or in cell culture supernatants.

The detection principle of this kit is based on HTRF™ technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1. The FcRn receptor biochemical binding assay is a competitive assay involving the extracellular ectodomain of FcRn that is biotinylated and bound to Terbium cryptate-labeled Streptavidin, and human IgG1 labelled with d2. The unlabeled antibody or Fc fused drug competes with the d2 labelled IgG1 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 (665 nm). Unlabeled antibodies present in the sample competes with the binding between the two HTRF detection solutions and thereby prevents FRET from occurring. The specific signal is inversely proportional to the antibody concentration.

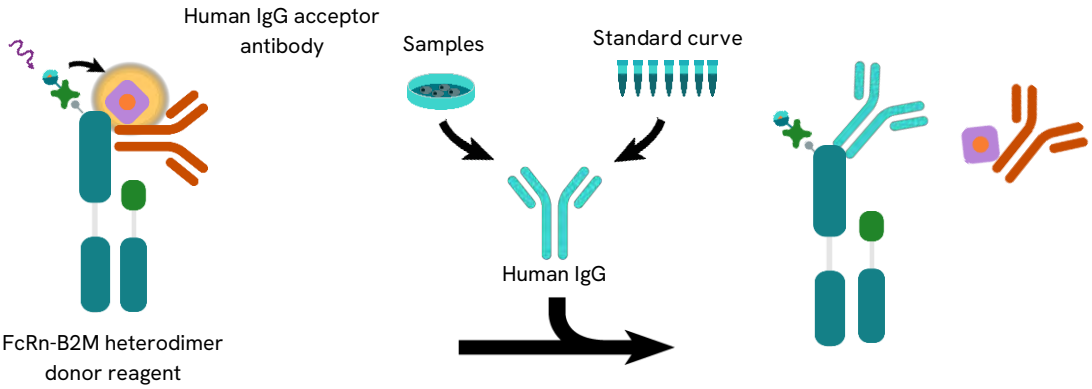
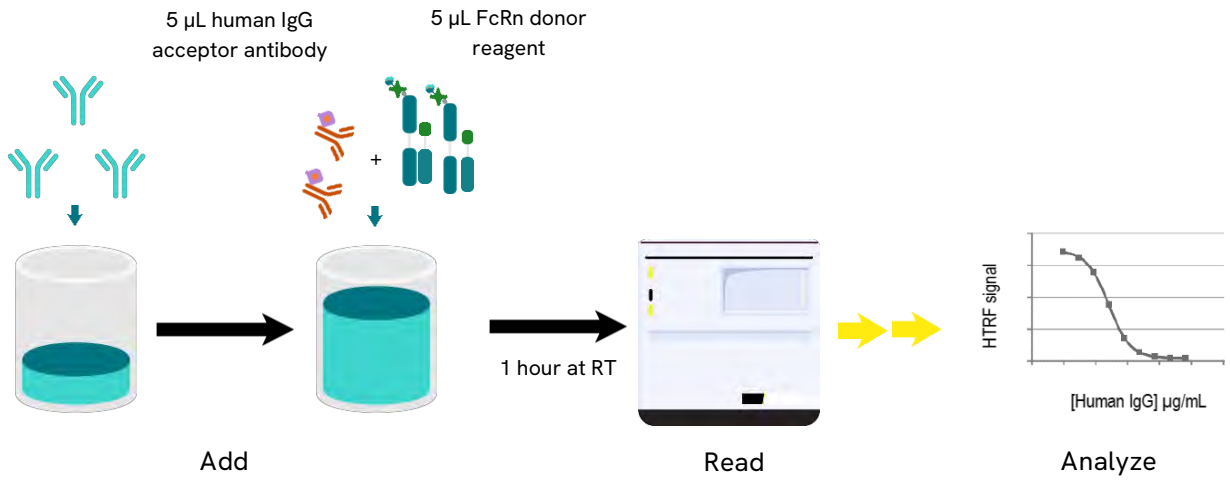


Figure 1: Principle of HTRF FcRn binding competitive assay.

PROTOCOL AT A GLANCE



Do not pre-mix the d2 and Cryptate solutions prior to dispensing.
Make sure to use the set-up for Tb Cryptate.

MATERIAL PROVIDED

KIT COMPONENTS	100 TESTS	500 TESTS	10,000 TESTS
Human IgG Standard Frozen	1 vial - 50 µL 6 mg/mL	1 vial - 50 µL 6 mg/mL	2 vials - 50 µL 6 mg/mL
FcRn - B2M heterodimer Tb Cryptate reagent Frozen	1 vial - 10 µL 50X	1 vial - 50 µL 50X	1 vial – 1 mL 50X
Human IgG d2 antibody Frozen	1 vial - 10 µL 50X	1 vial - 50 µL 50X	1 vial – 1mL 50X
Diluent** #10 Ready-to-use	1 vial - 10 mL	1 vial - 10 mL	1 vial - 100 mL
Detection Buffer*** Ready-to-use	1 vial - 1.2 mL	1 vial - 6 mL	1 vial - 100 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 100 tests and 500 tests respectively in 20 µL final volume. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent. The excess of biotin in the culture media may impair the kit assay performance.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents..

Purchase separately

- HTRF™-Certified Reader. Make sure the setup for Tb Cryptate is used.
  For a list of HTRF-compatible readers and set-up recommendations, please visit our website
- Small volume (SV) detection microplates - Use white plate only.
  For more information about microplate recommendations, please visit our website

STORAGE AND STABILITY

Kit

- Store the kit at -16°C.
- Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

Reagents

- If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
- Volume of Human IgG standard aliquots should not be under 10 µL.

REAGENT PREPARATION

Before you begin

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Human IgG standards (for standard curve) must be prepared in diluent or in the same medium as the samples. Cell culture medium can be alternative to the diluent. **Please note that RPMI medium is not suitable for the FcRn binding assay.**

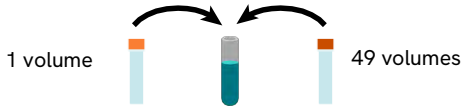
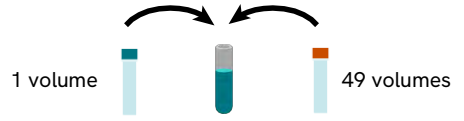
Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

To prepare reagent stock solutions

100 TESTS		500 & 10,000 TESTS
FcRn – B2M heterodimer Tb Cryptate reagent		
Thaw the FcRn - B2M heterodimer Tb Cryptate reagent. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below		Thaw the FcRn - B2M heterodimer Tb Cryptate reagent. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below
Human IgG d2 antibody		
Thaw the Human IgG d2 antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the Human IgG d2 antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Human IgG Standard		
Thaw the IgG standard solution in order to obtain a 6 mg/mL stock solution. Mix gently.		Thaw the IgG standard solution in order to obtain a 6 mg/mL stock solution. Mix gently.
Diluent		
The diluent is ready-to-use.		The diluent is ready-to-use.
Detection buffer		
The Detection buffer is ready-to-use.		

To prepare working antibody solutions

Each well requires 5 µL FcRn - B2M heterodimer Tb Cryptate reagent and 5 µL Human IgG d2 antibody. Prepare the two antibody solutions in separate vials.

100 TESTS	500 & 10,000 TESTS
FcRn – B2M heterodimer Tb Cryptate reagent	
	
Dilute 50-fold the 50X stock solution (thawed reagent) of FcRn Cryptate reagent stock solution with the Detection buffer #13: add 1 volume of Tb Cryptate heterodimer stock solution in 49 volumes of Detection buffer #13 (e.g., 5 µL of Tb Cryptate antibody stock solution + 245 µL of Detection Buffer #13)	Dilute 50-fold the 50X stock solution (thawed reagent) of FcRn Cryptate reagent stock solution with the Detection buffer #13: add 1 volume of Tb Cryptate heterodimer stock solution in 49 volumes of Detection buffer #13 (e.g., 20 µL of Tb Cryptate antibody stock solution + 980 µL of Detection Buffer #13).
Human IgG d2 antibody	
	
Dilute 50-fold the 50X stock solution (thawed reagent) of FcRn Cryptate reagent stock solution with the Detection buffer #13: add 1 volume of Tb Cryptate heterodimer stock solution in 49 volumes of Detection buffer #13 (e.g., 20 µL of Tb Cryptate antibody stock solution + 980 µL of Detection Buffer #13).	Dilute 50-fold the 50X stock solution (thawed reagent) of d2 antibody stock solution with the Detection buffer #13: add 1 volume of d2-antibody stock solution in 49 volumes of Detection buffer #13 (e.g., 20 µL of d2-antibody stock solution + 980 µL of Detection Buffer #13).

Do not pre-mix the d2 and the Tb Cryptate solutions prior to dispensing.

To prepare working standards solutions

- Each well requires 10 µL of standard.
- Dilute the standard stock solution serially with diluent #10 or appropriate medium
- In order to counteract any standard sticking, we recommend changing tips between each dilution

A recommended standard dilution procedure is listed and illustrated below

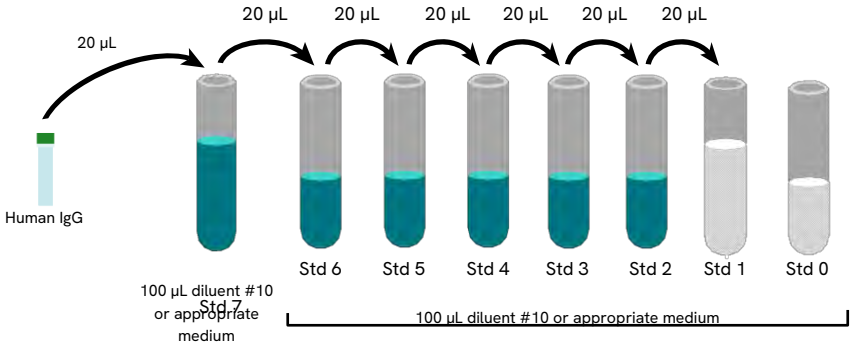
Dilute the standard stock solution 6-fold with diluent or cell culture medium; this yields the Standard Max solution (1mg/mL).

Dilute the standard stock solution 6-fold with diluent #10 or cell culture medium to prepare high standard (Std 7): e.g. take 20 µL of standard stock solution and add it to 100 µL of diluent #10 or cell culture medium. Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/6 serial dilutions as follows:

- Dispense 100 µL of diluent #10 or cell culture medium in each vial from Std 6 to Std 0.
- Add 20 µL of standard to 100 µL of diluent #10 or cell culture medium, mix gently and repeat the 1/6 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Positive control) is diluent #10 or appropriate medium alone.



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS (µg/mL)
Standard Stock solution	Thawed stock solution	6,000
Standard 7	20 µL standard stock + 100 µL diluent	1,000
Standard 6	20 µL standard 7 + 100 µL diluent	167
Standard 5	20 µL standard 6 + 100 µL diluent	28
Standard 4	20 µL standard 5 + 100 µL diluent	5
Standard 3	20 µL standard 4 + 100 µL diluent	0.77
Standard 2	20 µL standard 3 + 100 µL diluent	0.13
Standard 1	20 µL standard 2 + 100 µL diluent	0.02
Standard 0	150 µL diluent	0

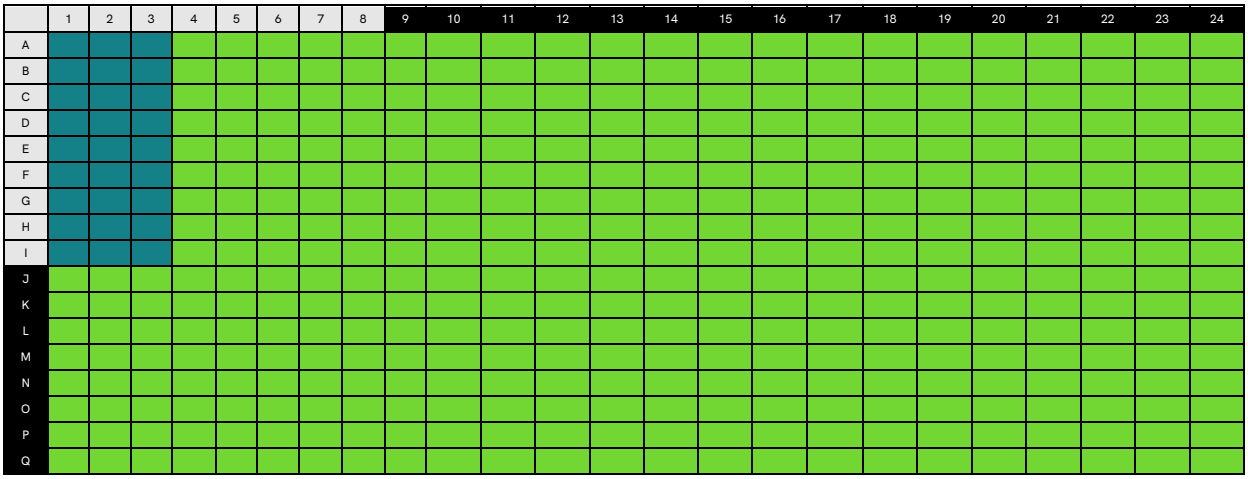
To prepare samples

- Each well requires 10 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent #10 or cell culture medium

ASSAY PROTOCOL

		NEGATIVE CONTROL OR CYPTATE CONTROL	STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 10 µL of diluent into each negative control well	Dispense 10 µL of each Human IgG standard (Std 0 - Std 7) into each standard well	Dispense 10 µL of each sample into each sample well
Step 2		Add 5 µL of detection buffer to all negative control wells	Add 5 µL human IgG acceptor antibody working solution to all wells	
Step 3			Add 5 µL FcRn donor reagent working solution to all wells	
Step 4			Seal the plate and incubate 1 hour at RT	
Step 5			Remove the plate sealer and read on an HTRF™ compatible reader	

	1	2	3	4	5	6
A	10 µL diluent (Negative control) 5 µL Detection Buffer #13 5 µL FcRn donor reagent	Repeat Well A1	Repeat Well A1	10 µL sample 1 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well A4	Repeat Well A4
B	10 µL Std 0 (Positive control) 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well B1	Repeat Well B1	10 µL sample 2 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well B4	Repeat Well B4
C	10 µL Std 1 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well C1	Repeat Well C1	10 µL sample 3 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well C4	Repeat Well C4
D	10 µL Std 2 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well D1	Repeat Well D1	10 µL sample 4 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well D4	Repeat Well D4
E	10 µL Std ... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well E1	Repeat Well E1	10 µL sample ... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well E4	Repeat Well E4
F	10 µL Std... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well F1	Repeat Well F1	10 µL sample ... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well F4	Repeat Well F4
G	10 µL Std... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well G1	Repeat Well G1	10 µL sample ... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well G4	Repeat Well G4
H	10 µL Std... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well H1	Repeat Well H1	10 µL sample ... 5 µL human IgG acceptor antibody 5 µL FcRn donor reagent	Repeat Well H4	Repeat Well H4



DATA REDUCTION & INTERPRETATION

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.

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

3) Calculate the % delta F which reflects the signal to background of the assay. The negative control plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

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

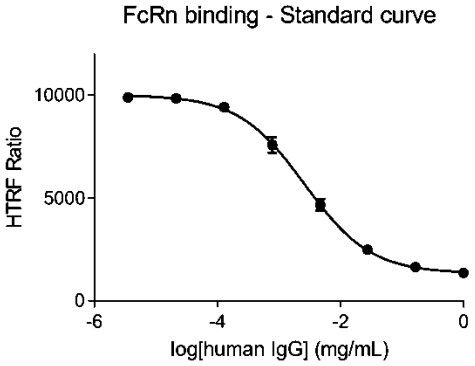
RESULTS

This data must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

Results may vary from one HTRF™ compatible reader to another.

The assay standard curve is created by plotting HTRF ratio versus the analyte concentration.

		Ratio (1)	CV% (2)
Negative control	Positive control	1 364	2.0%
Standard 0		10 191	0.0%
Standard 1	0.02 µg/mL	9 897	2.0%
Standard 2	0.13 µg/mL	9 846	1.0%
Standard 3	0.77 µg/mL	9 426	1.0%
Standard 4	5 µg/mL	7 585	5%
Standard 5	28 µg/mL	4 667	6.0%
Standard 6	167 µg/mL	2 487	3.0%
Standard 7	1,000 µg/mL	1 634	3%



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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