MANUAL

Technology: HTRF® Biomarker

HTRF Rabbit Fc Kit

Part number	6FRIGPEG	6FRIGPEH
Test size	500 tests	10,000 tests

Storage: ≤-60°C

Version: 03 **Date:** January 2024

ASSAY PRINCIPLE

This assay is intended for the quantitative determination of Rabbit IgG using the HTRF® technology. Rabbit IgG can be measured directly from cell supernatants or purified solutions.
As shown in the diagram below, Rabbit IgG is detected in a competitive assay format using 2 different antibodies, one labelled with Eu3+-Cryptate (donor) and the second with XL665 (acceptor).

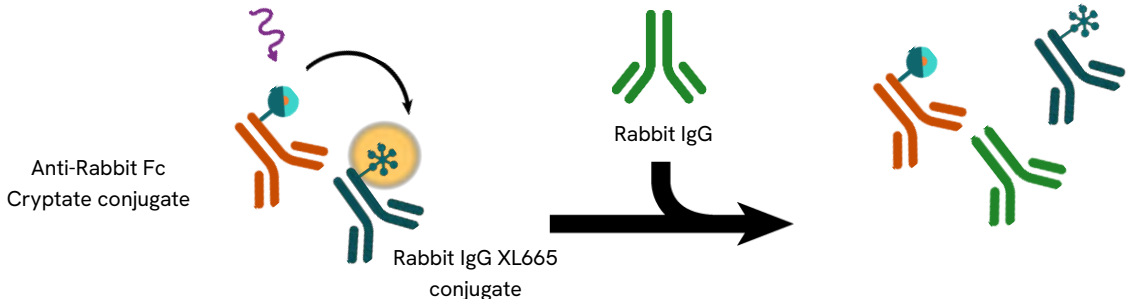
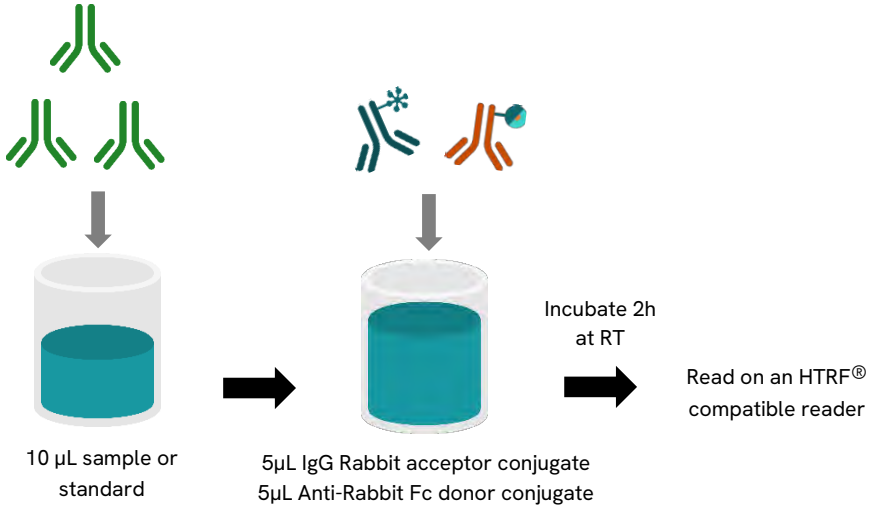


Figure 1: Principle of the assay.

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 Rabbit IgG present in the sample competes with the binding between the two conjugates and thereby prevents FRET from occurring. The specific signal is inversely proportional to Rabbit IgG concentration.

PROTOCOL AT A GLANCE



MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS		10,000 TESTS		STORAGE
Rabbit IgG Standard		10 µL/vial		10 µL/vial	≤-20°C
Rabbit IgG-XL665 conjugate		25 µL/vial		500 µL/vial	≤-60°C
Anti-Rabbit Fc-Eu^{3+}- Cryptate conjugate		25 µL/vial		500 µL/vial	≤-20°C
Diluent buffer #1		20 mL/vial		20 mL/vial	4°C to -20°C*
Detection buffer #2		13 mL/vial		2 x 50 mL/vial	4°C to -20°C*

* Diluent and Detection buffer are shipped frozen, but can be stored at 2-8°C in your premises.

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.

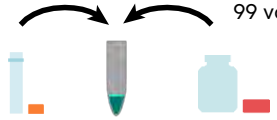
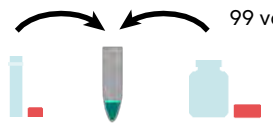
REAGENT PREPARATION

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 quality.
For an accurate quantitative determination of sample, dilution must be carried out with the medium used for preparing the samples (i.e. diluent, culture medium or any other compatible medium).
Standard and conjugates may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of standard and conjugates into disposable plastic vials for storage at –20°C or below.

- Thaw all reagents at room temperature.
- Prepare the working solutions from stock solutions by following the instructions below.

Preparation of conjugate working solutions

Determine the amount of antibodies needed for the experiment. Each well requires 5 µL of each antibody.

500 TESTS	10,000 TESTS
Rabbit IgG-XL665 conjugate	
1 volume 99 volumes 	1 volume 99 volumes 
Prepare a 100X diluted solution using the conjugate buffer: e.g. take 25 µL of conjugate stock solution and add it to 2475 µL of conjugate buffer.	Prepare a 100X diluted solution using the conjugate buffer: e.g. take 500 µL of conjugate stock solution and add it to 49,5 mL of conjugate buffer.
Anti-Rabbit Fc-Eu^{3+}-Cryptate conjugate	
1 volume 99 volumes 	1 volume 99 volumes 
Prepare a 100X diluted solution using the conjugate buffer: e.g. take 25 µL of conjugate stock solution and add it to 2475 µL of conjugate buffer.	Prepare a 100X diluted solution using the conjugate buffer: e.g. take 500 µL of conjugate stock solution and add it to 49,5 mL of conjugate buffer.

Standard curve preparation

Determine how many samples and replicates to be tested. Each well requires 10 µL of standard or sample.
NB: If the sample to test is a cell supernatant, replace the diluent by culture medium.

STANDARD	PREPARATION	WORKING CONCENTRATION (ng/mL)
Standard 8	4 µL standard stock solution + 396 µL diluent	80 000
Standard 7	100 µL Std 8 + 300 µL diluent	20 000
Standard 6	100 µL Std 7 + 300 µL diluent	5 000
Standard 5	100 µL Std 6 + 300 µL diluent	1 250
Standard 4	100 µL Std 5 + 300 µL diluent	312
Standard 3	100 µL Std 4 + 300 µL diluent	78
Standard 2	100 µL Std 3 + 300 µL diluent	19.5
Standard 1	100 µL Std 2 + 300 µL diluent	4.9
Standard 0	300 µL diluent	0

Dilute 100-fold the standard stock solution using diluent; this yields the high standard (Std 8: 80 000 ng/mL) for the top of the curve. In practice:

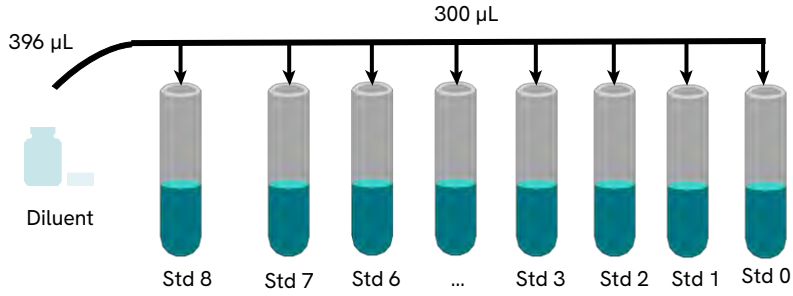
- e.g. add 396 µL of diluent to 4 µL of standard stock solution. Mix gently.

Use the high standard (Std 8) to prepare the standard curve using 1/4 serial dilutions as follows:

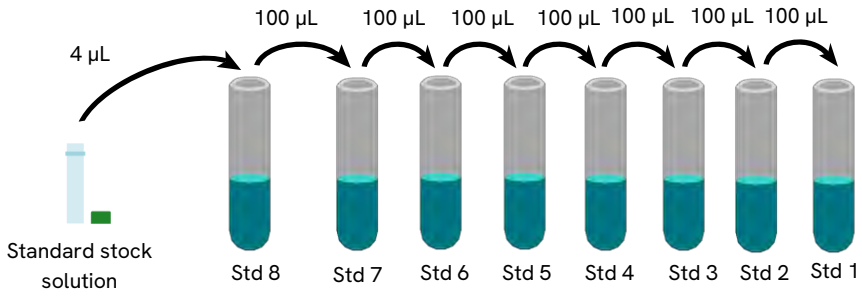
- Dispense 300 µL of diluent in each vial from Std 7 to Std 1.
- Add 100 µL of standard to 300 µL of diluent, mix gently and repeat the 1/4 serial dilution to make standard solutions: 20 000, 5 000, 1 250, 312, 78, 19.5 and 4.9 ng/mL.

This will create 8 standards for the analyte. Std 0 (Positive control) is diluent alone.

Step 1: dispense diluent in each vial

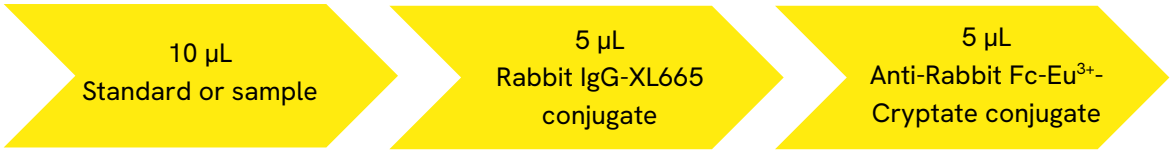


Step 2: dilute standards



ASSAY PROTOCOL

Dispense the reagents in the following order:



Carefully follow the order of dispensing and DO NOT pre-mix the two conjugates.

- Cover the plate with a plate sealer.
- Incubate at RT 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			
	Positive control	Negative control or cryptate control	Buffer control	Sample / Std
	used to calculate the Signal Max	*used to calculate the delta F% and to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Sample / Std	-	-	-	10 µL
Diluent buffer #1	10 µL	10 µL	10 µL	-
Rabbit IgG-XL665 conjugate	5 µL	-	-	5 µL
Anti-Rabbit Fc-Eu^{3+}-Cryptate conjugate	5 µL	5 µL	-	5 µL
Conjugate buffer #2	-	5 µL	10 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.

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

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.

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

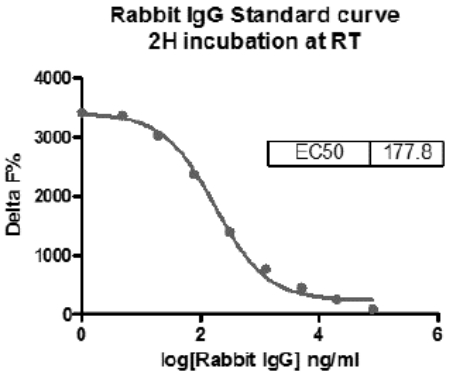
For more information about data reduction, please visit our website.

To determine sample concentration, we recommend to use a log scale for the Mouse IgG concentrations and analyze the data with the sigmoidal dose response curve with variable slope.

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 (ng/mL)		Ratio (1)	CV % (2)	Delta F% (3)
Negative control		421	0.5%	0
Standard 0	Positive control	14 827	1.3%	3 419
Standard 1	4.9	14 570	1.0%	3 358
Standard 2	19.5	13 187	0.1%	3 030
Standard 3	78	10 451	0.8%	2 380
Standard 4	312	6 312	1.6%	1 398
Standard 5	1 250	3 646	0.9%	765
Standard 6	5 000	2 304	1.3%	447
Standard 7	20 000	1 483	0.5%	252
Standard 8	80 000	791	0.7%	88



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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