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Rabbit Fc Kit
500 tests

For in vitro research use only
Reagent storage temperature: -20°C or below

www.cisbio.com

Packaging details:

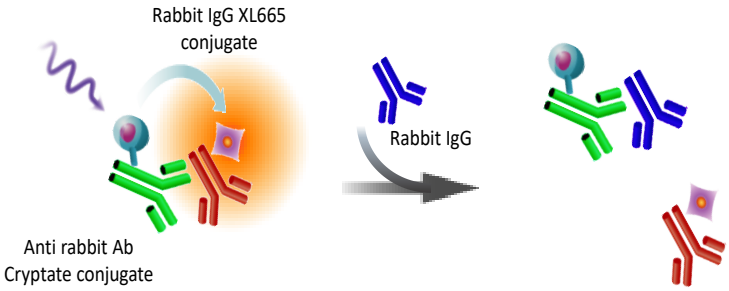
	384-well low volume plate (20 µl)
6FRIGPEG	500 tests

Product information

Document reference: 6FRIGPEG-Rev02-Dec.2020

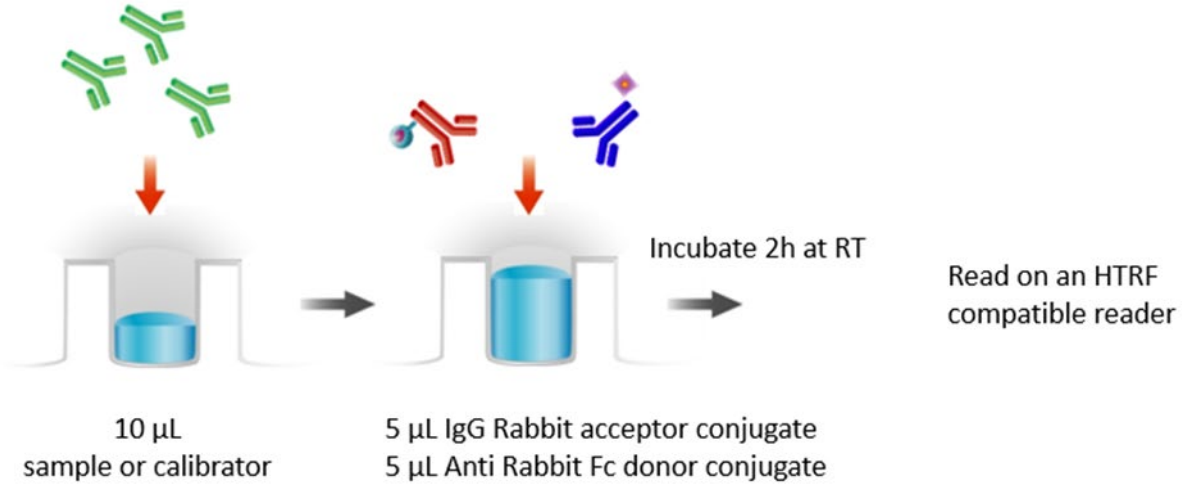
1. Assay description

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 to the right, Rabbit IgG is detected in a competitive assay format using 2 different antibodies, one labelled with Eu^{3+}-Cryptate (donor) and the second with XL665 (acceptor).
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).

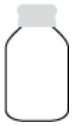


The Rabbit IgG present in the sample competes with the binding between the two conjugates and thereby prevents FRET from occurring. The specific signal is inversely proportional to Rabbit IgG concentration.

2. Protocol at a glance



3. HTRF® reagents

	Rabbit IgG Standard	Rabbit IgG-XL665 conjugate	Anti-Rabbit Fc-Eu³⁺-Cryptate conjugate	Diluent buffer	Detection buffer
	 green cap	 blue cap	 orange cap	 white cap	 red cap
Stock solution	10 µL/vial 8 mg/mL	25 µL/vial	25 µL/vial	20 mL/vial	13 mL/vial
Storage	-20°C or below	-20°C or below	-20°C or below	4°C to -20°C*	4°C to -20°C*

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

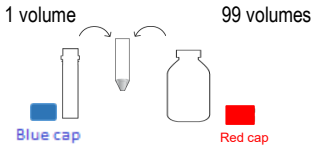
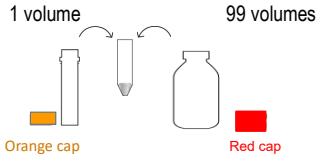
4. Reagent preparation

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 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 (§3) by following the instructions below.

4.1. Preparation of antibody working solutions

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

Rabbit IgG-XL665 conjugate	Anti-Rabbit Fc-Eu³⁺-Cryptate conjugate
	
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 25 µL of conjugate stock solution and add it to 2475 µL of conjugate buffer.

4.2. 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.

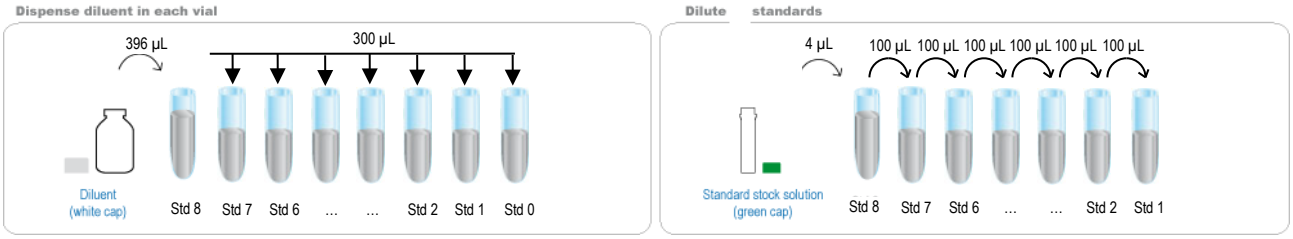
Standards	Working concentration (ng/mL)	Preparation
Std 8	80 000	4 µL standard stock solution + 396 µL diluent
Std 7	20 000	100 µL Std 8 + 300 µL diluent
Std 6	5 000	100 µL Std 7 + 300 µL diluent
Std 5	1 250	100 µL Std 6 + 300 µL diluent
Std 4	312	100 µL Std 5 + 300 µL diluent
Std 3	78	100 µL Std 4 + 300 µL diluent
Std 2	19.5	100 µL Std 3 + 300 µL diluent
Std 1	4.9	100 µL Std 2 + 300 µL diluent
Std 0	0	300 µL diluent

A recommended standard dilution procedure is listed and illustrated below.

→ *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.



5. Assay protocol

Dispense the reagents in the following order:

10 µL Standard or sample → 5 µL Rabbit IgG-XL665 conjugate → 5 µL Anti-Rabbit Fc-Eu^{3+}-cryptate conjugate

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 and,
→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

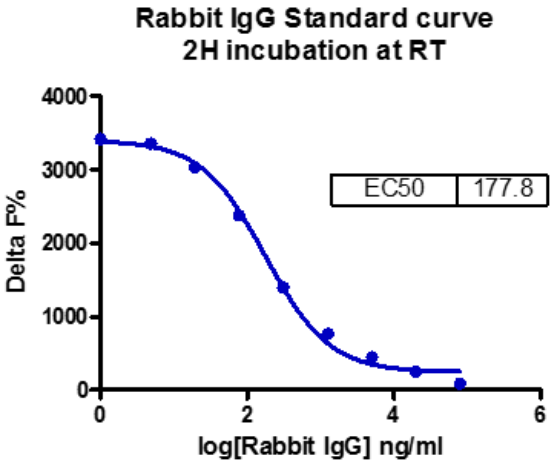
For more information about HTRF® compatible readers, please visit our website at: www.cisbio.com/compatible-readers

		Assay 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	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	-	5 µL	10 µL	-

6. Data reduction

These data must not be substituted for that obtained in the laboratory and should be considered only as an example (obtained on an HTRF® compatible reader). Results may vary from one HTRF® compatible reader to another.
The assay standard curve is drawn up by plotting delta F% versus the analyte concentration:

Standards ng/mL	Ratio (1)	CV % (2)	Delta F % (3)
Negative control	421	0.5%	0
Std 0 – Positive control	14827	1.3%	3419
Std 1 – 4.9	14570	1.0%	3358
Std 2 – 19.5	13187	0.1%	3030
Std 3 – 78	10451	0.8%	2380
Std 4 – 312	6312	1.6%	1398
Std 5 – 1250	3646	0.9%	765
Std 6 – 5 000	2304	1.3%	447
Std 7 – 20 000	1483	0.5%	252
Std 8 – 80 000	791	0.7%	88



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.
Delta F % (3)	$\frac{\text{Ratio}_{\text{standard or sample}} - \text{Ratio}_{\text{Negative control}}}{\text{Ratio}_{\text{Negative control}}} \times 100$	Reflects the signal to background of the assay. The negative control plays the role of an internal assay control.
For more information about data reduction, please visit our website at: www.cisbio.com/htrf-ratio-and-data-reduction		

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

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