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Europe and other countries +33(0)466-796-705
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PerkinElmer For the Better | cisbio

Mouse Fc Kit
10.000 tests

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

www.cisbio.com

Packaging details:

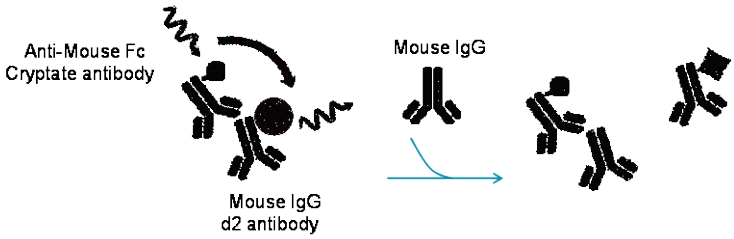
	384-well low volume plate (20 µl)
6FMIGPEH	10.000 tests

Product information

Document reference: 6FMIGPEH-Rev03-Nov.2020

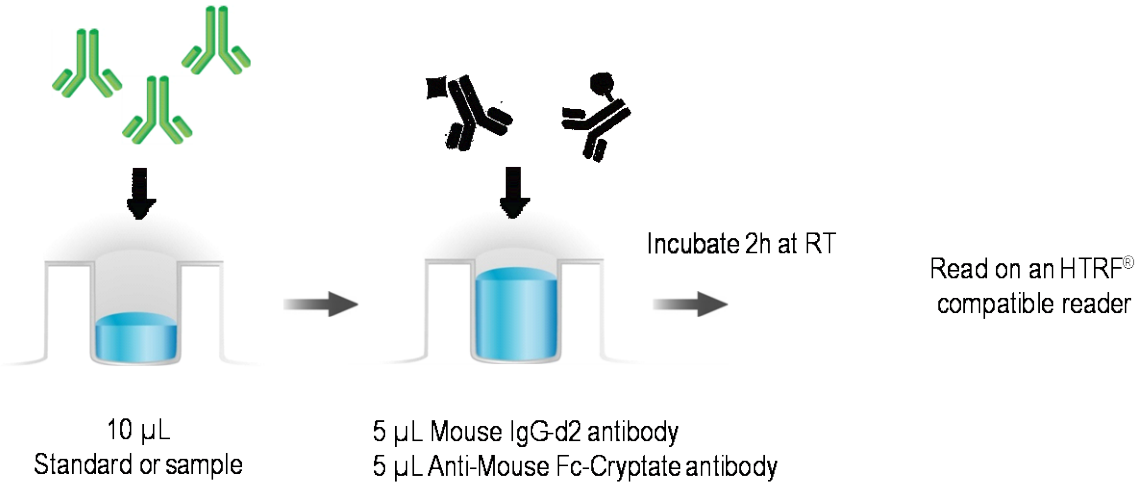
1. Assay description

This assay is intended for the quantitative determination of Mouse IgG using the HTRF® technology. Mouse IgG can be measured directly from cell supernatants or purified solutions.
As shown in the diagram to the right, Mouse IgG is detected in a competitive assay format using 2 different antibodies, one labelled with Eu^{3+}-Cryptate (donor) and the second with d2 (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).

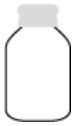


The Mouse IgG present in the sample competes with the binding between the two antibodies and thereby prevents FRET from occurring. The specific signal is inversely proportional to Mouse IgG concentration.

2. Protocol at a glance



3. HTRF® reagents

	Mouse IgG Standard	Mouse IgG-d2 antibody	Anti-Mouse Fc-Eu³⁺-Cryptate antibody	Diluent	Detection buffer #3
					
Stock solution	25 µL/vial 4.35 mg/mL	1000 µL/vial	1000 µL/vial	20 mL/vial	105 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.

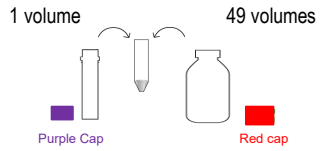
4. Reagent preparation

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate antibodies 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 antibodies may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended to dispense remaining stock solutions of standard and antibodies 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 antibody.

Mouse IgG-d2 antibody	Anti-Mouse Fc-Eu³⁺-Cryptate antibody
	
Prepare a 50X diluted solution using the detection buffer #3: e.g. take 1000 µL of antibody stock solution and add it to 49 mL of detection buffer #3.	Prepare a 50X diluted solution using the detection buffer #3: e.g. take 1000 µL of antibody stock solution and add it to 49 mL of detection buffer #3.

4.2. Standard curve preparation

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

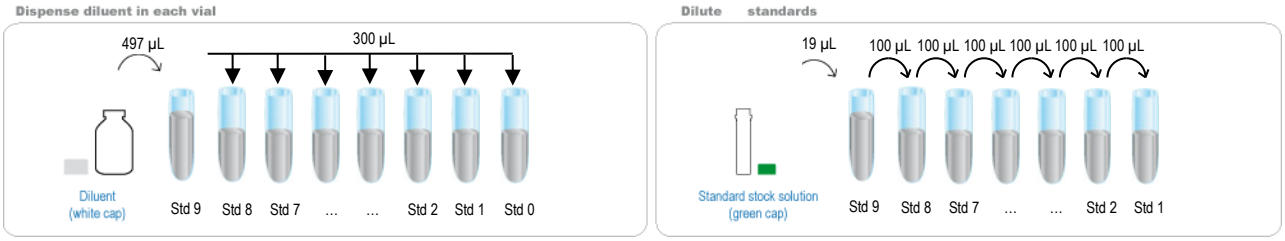
Standards	Working concentration (ng/mL)	Preparation
Std 9	160 000	19 µL standard stock solution + 497 µL diluent
Std 8	40 000	100 µL Std 8 + 300 µL diluent
Std 7	10 000	100 µL Std 7 + 300 µL diluent
Std 6	2 500	100 µL Std 6 + 300 µL diluent
Std 5	625	100 µL Std 5 + 300 µL diluent
Std 4	156.25	100 µL Std 4 + 300 µL diluent
Std 3	39.06	100 µL Std 3 + 300 µL diluent
Std 2	9.76	100 µL Std 2 + 300 µL diluent
Std 1	2.44	100 µL Std 1 + 300 µL diluent
Std 0	0	300 µL diluent

A recommended standard dilution procedure is listed and illustrated below.

→ *Dilute 27.19-fold the standard stock solution using diluent; this yields the high standard (Std 9: 160 000 ng/mL) for the top of the curve. In practice:*
- *e.g. add 497 µL of diluent to 19 µL of standard stock solution. Mix gently.*

→ *Use the high standard (Std 9) to prepare the standard curve using 1/4 serial dilutions as follows:*
- *Dispense 300 µL of diluent in each vial from Std 8 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: 40 000, 10 000, 2 500, 625, 156.25, 39.06, 9.76 and 2.44, ng/mL.*

This will create 9 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 Mouse IgG-d2 antibody → 5 µL Anti-Mouse Fc-Eu^{3+}-Cryptate antibody

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

→ 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.

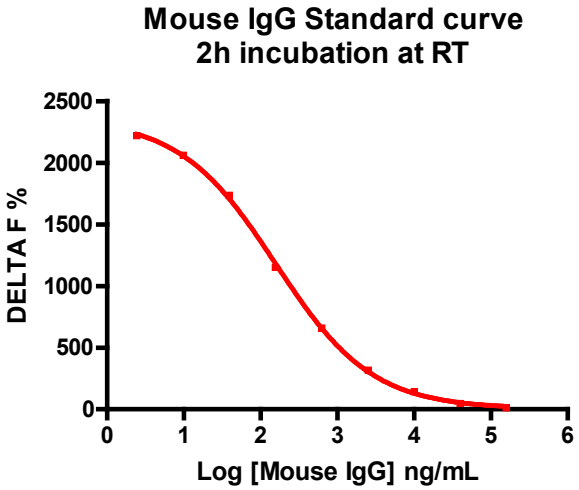
For more information about HTRF® compatible readers, please visit our website at: www.cisbio.com/compatible-readers

		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	10 µL	10 µL	10 µL	-
Mouse IgG-d2 conjugate	5 µL	-	-	5 µL
Anti-Mouse Fc-Eu^{3+}-Cryptate conjugate	5 µL	5 µL	-	5 µL
Detection buffer #3	-	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	498	2.38%	0
Std 0 – Positive control	12 125	1.30%	2335
Std 1 – 2.44	11 550	1.88%	2220
Std 2 – 9.76	10 761	0.92%	2062
Std 3 – 39.06	9 143	0.61%	1737
Std 4 – 156.25	6 227	0.83%	1151
Std 5 – 625	3 783	1.12%	660
Std 6 – 2 500	2 081	1.52%	318
Std 7 – 10 000	1 205	1.83%	142
Std 8 – 40 000	731	1.01%	47
Std 9 – 160 000	552	2.62%	11



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 Mouse IgG concentrations and analyze the data with the sigmoidal dose response curve with variable slope.