MANUAL

Technology: HTRF®

HTRF Human IgG Lambda kit

Part number	64LAMPEG	64LAMPEH
Test size	500 tests	10,000 tests

Version: 03 **Date:** January 2024

ASSAY PRINCIPLE

This assay is intended for the measurement of human (h) IgG Lambda light chain of all types of IgG (IgG1, IgG2, IgG3 and IgG4) using the HTRF® technology.
As shown here, (h)IgG Lambda light chain is detected in a sandwich assay format using 2 different specific antibodies. The anti-(h)-IgG Lambda antibody is labelled with Eu^{3+}-Cryptate (donor) and the anti(h)-Fc antibody is labelled with d2 (acceptor).

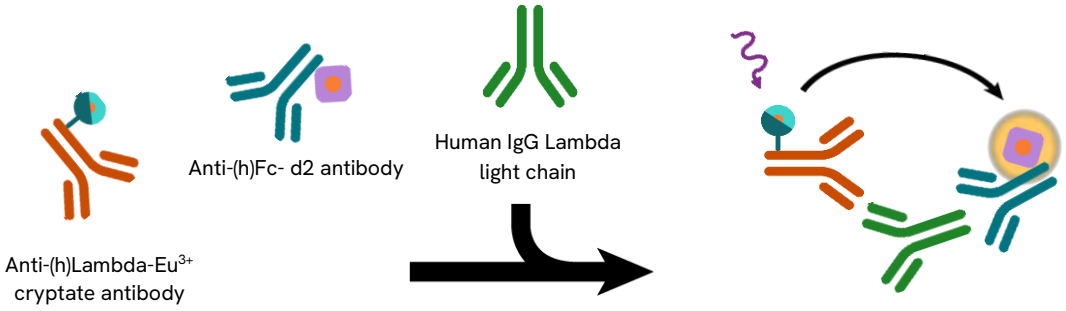
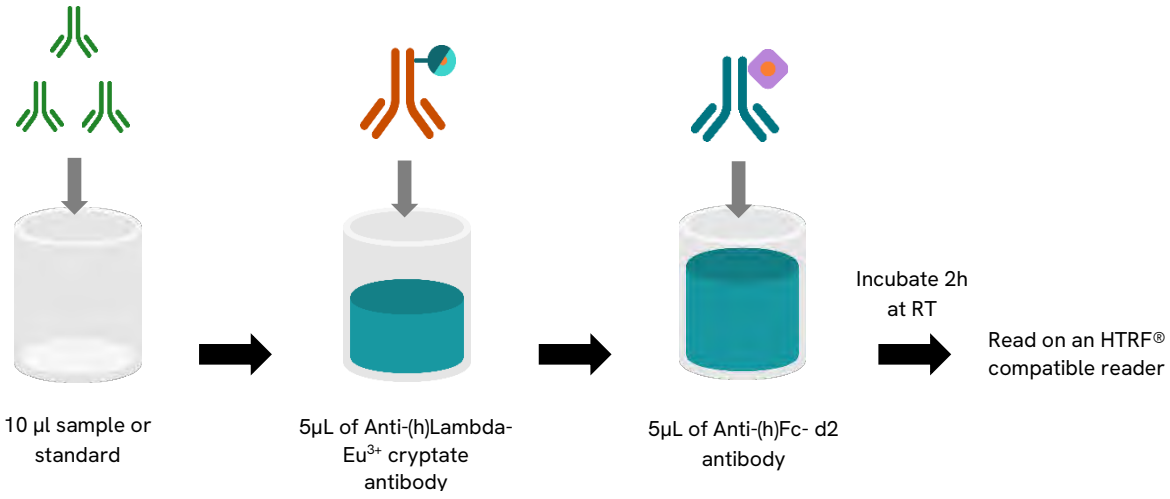


Figure 1: Principle of the assay.

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 two antibodies bind to the (h)IgG Lambda present in the sample, thereby generating FRET. The specific signal modulates positively in proportion to (h)IgG Lambda.

PROTOCOL AT A GLANCE



MATERIAL PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS			10,000 TESTS		
Standard (h)IgGs	≤-20°C		green cap	50 µl/vial 4 µg/mL		green cap	50 µl/vial 4 µg/mL
Anti-(h)Fc-d2 antibody	≤-20°C		blue cap	50 µl/vial		purple cap	1,000 µl/vial
Anti-(h)IgG Lambda-Eu^{3+}-Cryptate antibody	≤-20°C		orange cap	50 µl/vial		orange cap	1,000 µl/vial
Diluent	4°C to -20°C*		transparent cap	20 ml/vial		transparent cap	20 ml/vial
Detection buffer #3	4°C to -20°C*		transparent cap	7 ml/vial		red cap	105 ml/vial

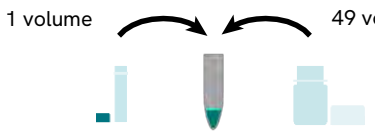
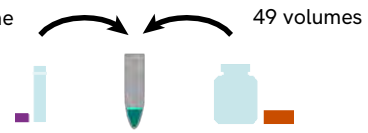
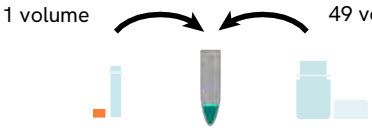
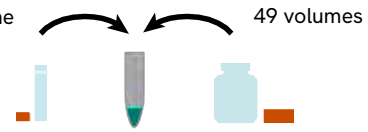
* Diluent and Detection buffer are shipped frozen, but can then be stored at 2-8°C

REAGENT PREPARATION

Thaw all reagents at room temperature, allow them to warm up (caution: take buffers' thawing time into account). Prepare the working solutions from stock solutions by following the instructions below.

Preparation of antibody working solutions

Determine the amounts of each detection reagent needed for the experiment. Each well requires 5µL of each detection reagent.

500 TESTS KIT	10,000 TESTS KIT
Anti-(h)Fc-d2 antibody	
	
Prepare a 50X diluted solution using the detection buffer #3: e.g. take 50 µL of antibody stock solution and add it to 2450 µL 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.
Anti-(h)IgG Lambda-Eu^{3+}-Cryptate antibody	
	
Prepare a 50X diluted solution using the detection buffer #3: e.g. take 50 µL of antibody stock solution and add it to 2450 µL 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

Be careful, working solution preparation may differ between the 500 and the 10,000 data point kits.

Standard curve preparation

Determine how many standard levels and replicates to be tested. Each well requires 10 µL of standard.

A whole IgG standard is provided with this kit.

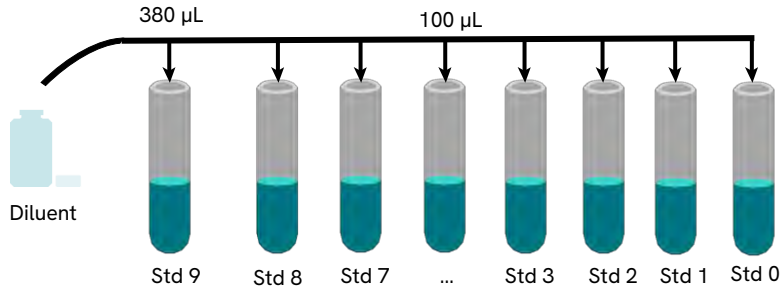
For a more specific and quantitative calibration, we recommend the use of an appropriate IgG subtype: IgG1, IgG2, IgG3 or IgG4.

STANDARD	PREPARATION	WORKING CONCENTRATION (ng/mL)
Standard 9	20 µL of standard stock solution + 380 µL diluent	200
Standard 8	100µl Std 9 + 100µl diluent	100
Standard 7	100µl Std 8 + 100µl diluent	50
Standard 6	100µl Std 7 + 100µl diluent	25
Standard 5	100µl Std 6 + 100µl diluent	12.5
Standard 4	100µl Std 5 + 100µl diluent	6.25
Standard 3	100µl Std 4 + 100µl diluent	3.1
Standard 2	100µl Std 3 + 100µl diluent	1.6
Standard 1	100µl Std 2 + 100µl diluent	0.8
Standard 0	100µl diluent	0

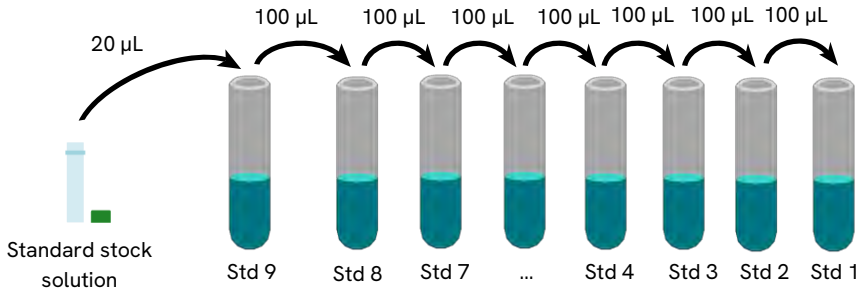
A recommended standard dilution procedure is listed below, and illustrated on the next page.

- Dilute the standard stock solution 20-fold with diluent. This yields the high standard (Std 9) for the top of the curve (200ng/mL). In practice:
  - e.g. take 20µL of the standard stock solution and add it to 380 µL of diluent. Mix gently.
- Use the high standard (Std 9) to prepare the standard curve using 1/2 serial dilutions as follows:
  - Dispense 100µL of diluent in each vial from Std 8 to Std 1.
  - Add 100µL of standard 9 to 100µL of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: 100, 50, 25, 12.5, 6.25, 3.1, 1.6, 0.8 ng/mL. This will create 9 standards for the analyte.
  - Std 0 (negative control) is diluent alone.

Step 1: dispense diluent in each vial.



Step 2: dilute standards

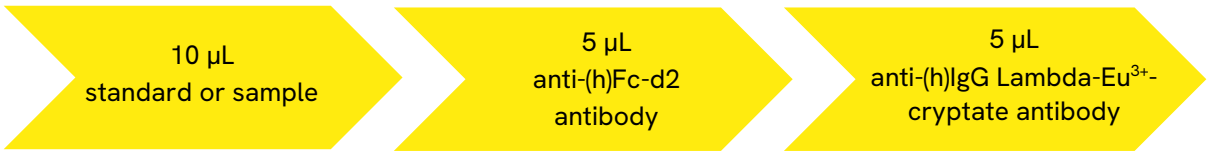


Recommendations:

- 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's 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.

ASSAY PROTOCOL

Dispense the reagents in the following order:



The 2 HTRF® antibodies can be pre-mix JUST PRIOR to dispensing: DO NOT store the pre-mix solution.

- Cover the plate with a plate sealer.
- Incubate at room temperature 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 HTRF certified reader

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

	Assays controls			
	Negative control	Cryptate control	Buffer control	Sample / Std
	used to calculate the delta F%	*used to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Sample / Std	-	-	-	10 µL
Diluent	10 µL	10 µL	10 µL	-
Anti-(h)Fc-d2 antibody	5 µL	-	-	5 µL
Anti-(h)IgG Lambda-Eu^{3+}-Cryptate antibody	5 µL	5 µL	-	5 µL
Detection buffer#3	-	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.

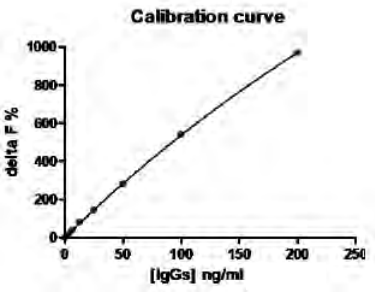
RESULTS

This 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.

The assay standard curve is drawn up by plotting delta F% versus the analyte concentration:

Standard ng/ml		Ratio (1)	CV % (2)	Delta F%
Standard 0	Negative controle	528	4,8%	0%
Standard 1	0.8	532	3,6%	1%
Standard 2	1.6	576	1,2%	9%
Standard 3	3.1	603	2,1%	14%
Standard 4	6.25	718	0,0%	36%
Standard 5	12.5	956	2,4%	81%
Standard 6	25	1286	2,2%	143%
Standard 7	50	2007	2,9%	280%
Standard 8	100	3390	0,3%	541%
Standard 9	200	5645	1,6%	968%



ASSAY CHARACTERISTICS

Cross-reactivity

	Cross-reactivity %
Human Lambda	100
Human Kappa	0
Mouse Lambda	0
Human IgM	0

Detection limit

Human Lambda (IgG1) = 0.4 ng/mL

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.

Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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