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Human IgG Lambda Kit
500 tests

Packaging details:

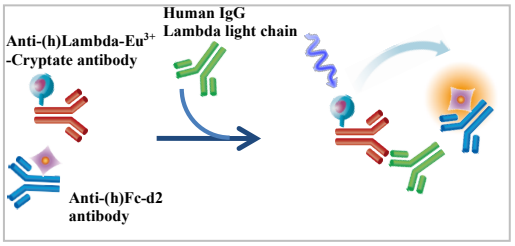
	384-well low volume plate (20 µl)
64LAMPEG	500 tests

www.cisbio.com

Product information

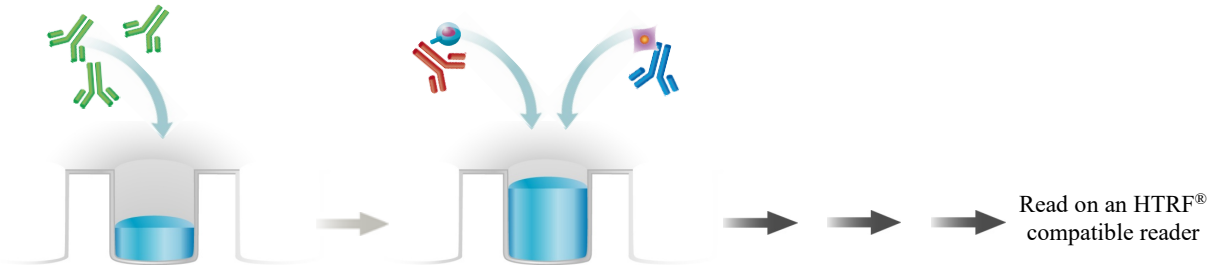
Document reference: 64LAMPEG-Rev02-Sept.2019

1. ASSAY DESCRIPTION



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).
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.

2. PROTOCOL AT A GLANCE



3. HTRF® REAGENTS

	Standard (h)IgGs	Anti-(h)Fc-d2 antibody	Anti-(h)IgG Lambda-Eu^{3+}-Cryptate antibody	Diluent	Detection buffer #3
Stock solution	50 µL/vial 4 µg/mL	50 µL/vial	50 µL/vial	20 mL/vial	7 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*

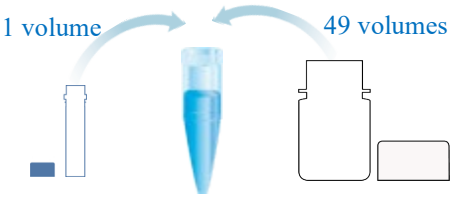
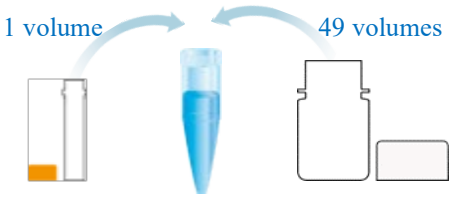
* Diluent and Detection buffer are shipped frozen, but can then be stored at 2-8°C

4. 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 (§3) by following the instructions below.

4.1. Preparation of antibody working solutions

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

Anti-(h)Fc-d2 antibody	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 50 µL of antibody stock solution and add it to 2450 µL of detection buffer #3

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

4.2. Standard curve preparation

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

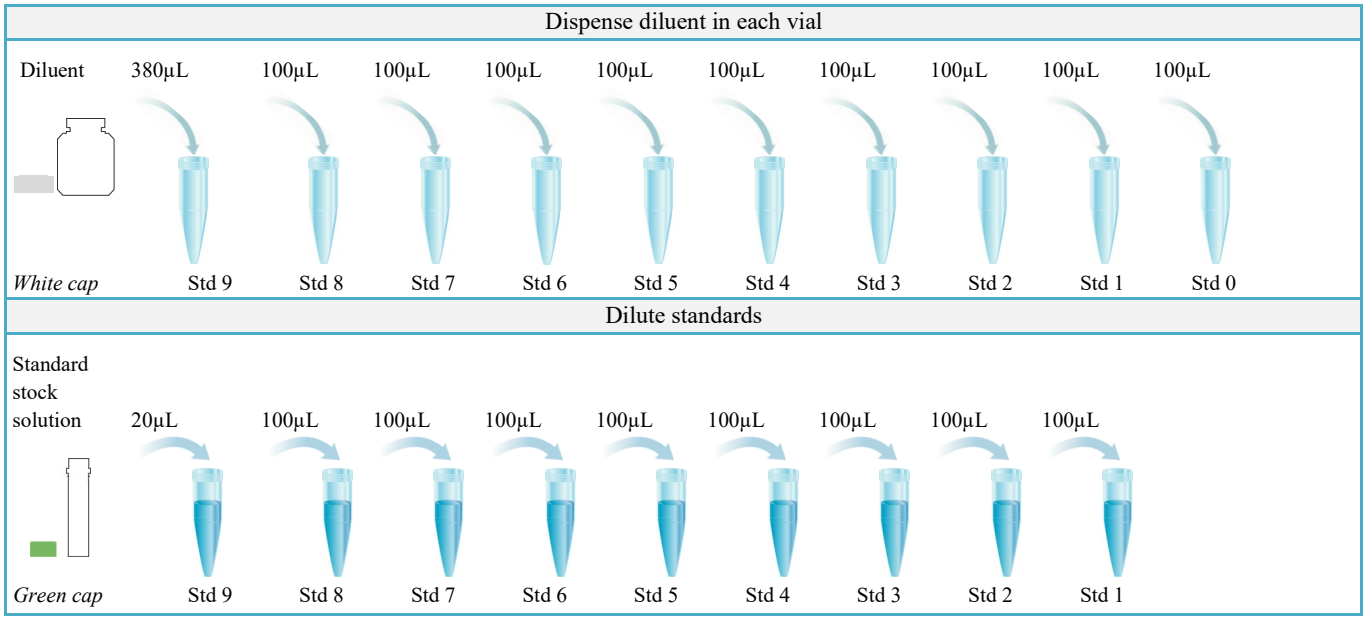
Standards	Working concentration (ng/mL)	Preparation
Std 9	200	20µL of Std stock solution + 380µL diluent
Std 8	100	100µl Std 9 + 100µl diluent
Std 7	50	100µl Std 8 + 100µl diluent
Std 6	25	100µl Std 7 + 100µl diluent
Std 5	12.5	100µl Std 6 + 100µl diluent
Std 4	6.25	100µl Std 5 + 100µl diluent
Std 3	3.1	100µl Std 4 + 100µl diluent
Std 2	1.6	100µl Std 3 + 100µl diluent
Std 1	0.8	100µl Std 2 + 100µl diluent
Std 0	0	100µl diluent

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 380µL 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 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.



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.

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

5. 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

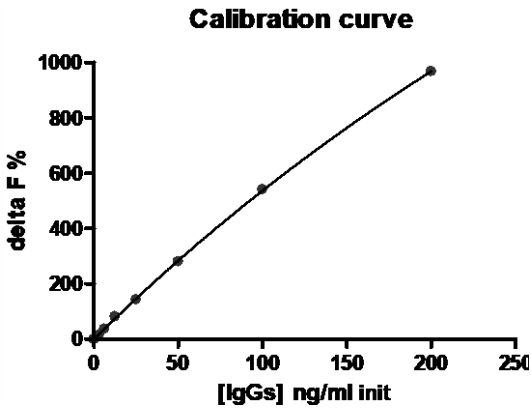
⇒ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader (more information about compatible reader at www.cisbio.com/readers)

	Assay 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	-	-	-	10µL
Diluent	10µL	10µL	10µL	-
Anti-(h)Fc-d2 antibody	5µL	-	-	5µL
Anti-(h)IgG Lambda-Eu^{3+}-Cryptate antibody	5µL	5µL		5µL
Detection buffer #3	-	5µL	10µL	-

6. DATA REDUCTION

This data must not be substituted for that obtained in the laboratory and should be considered only as an example *(readouts on PHERAstar^{plus})*. 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 % (3)
Std 0 – Negative control	528	4.8%	0%
Std 1 – 0.8	532	3.6%	1%
Std 2 – 1.6	576	1.2%	9%
Std 3 – 3.1	603	2.1%	14%
Std 4 – 6.25	718	0.0%	36%
Std 5 – 12.5	956	2.4%	81%
Std 6 - 25	1286	2.2%	143%
Std 7 - 50	2007	2.9%	280%
Std 8 - 100	3390	0.3%	541%
Std 9 - 200	5645	1.6%	968%



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/data-reduction		

7. ASSAY CHARACTERISTICS

7.1. Cross-reactivity

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

7.2. Detection limit

Human Lambda (IgG1) = 0.4ng/mL

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