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Human IgG Kappa kit
500 tests

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

www.cisbio.com

Product information

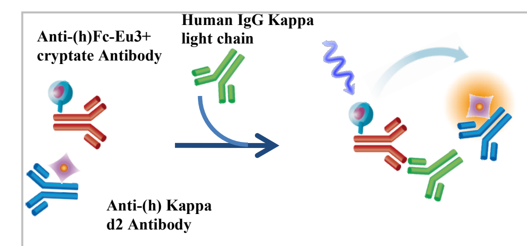
Document reference : 64KAPPEG – Rev3 – December 2020

Packaging details :

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

1. ASSAY DESCRIPTION

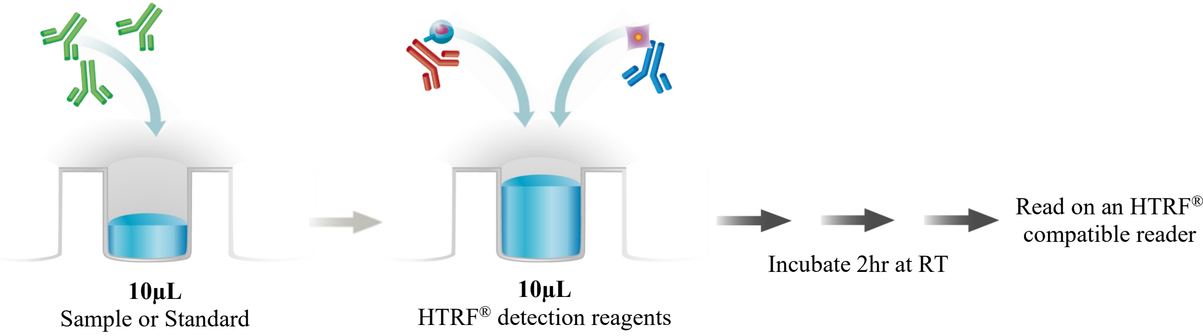
This assay is intended for the measurement of human (h) IgG Kappa light chain of all types of IgG (IgG1, IgG2, IgG3 and IgG4) using the HTRF® technology.



As shown here, (h)IgG Kappa light chain is detected in a sandwich assay format using 2 different specific antibodies. The anti-(h)-IgG Kappa antibody is labelled with d2 (acceptor) and the anti(h)-Fc antibody is labelled with Eu^{3+}-Cryptate (donor).

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

2. PROTOCOL AT A GLANCE



2. HTRF REAGENTS

	Standard (h)IgGs	Anti-(h) IgG Kappa d2 antibody	Anti-(h) Fc Eu3+ Cryptate antibody	Diluent	Detection buffer#3
Stock solution	50µl/vial 4µg/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*

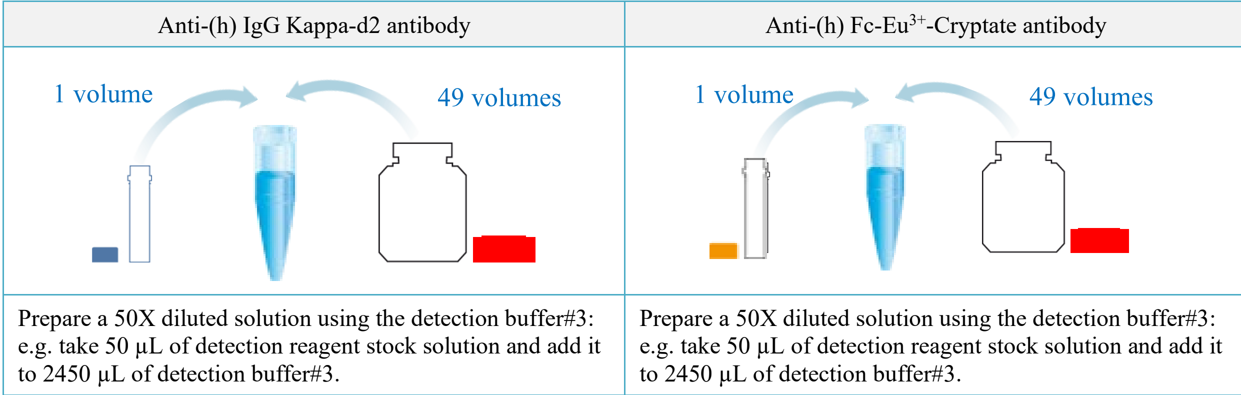
* Diluent and Detection buffer are shipped frozen, but can then be stored at 2-8°C

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



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

4.2. Standard curve preparation

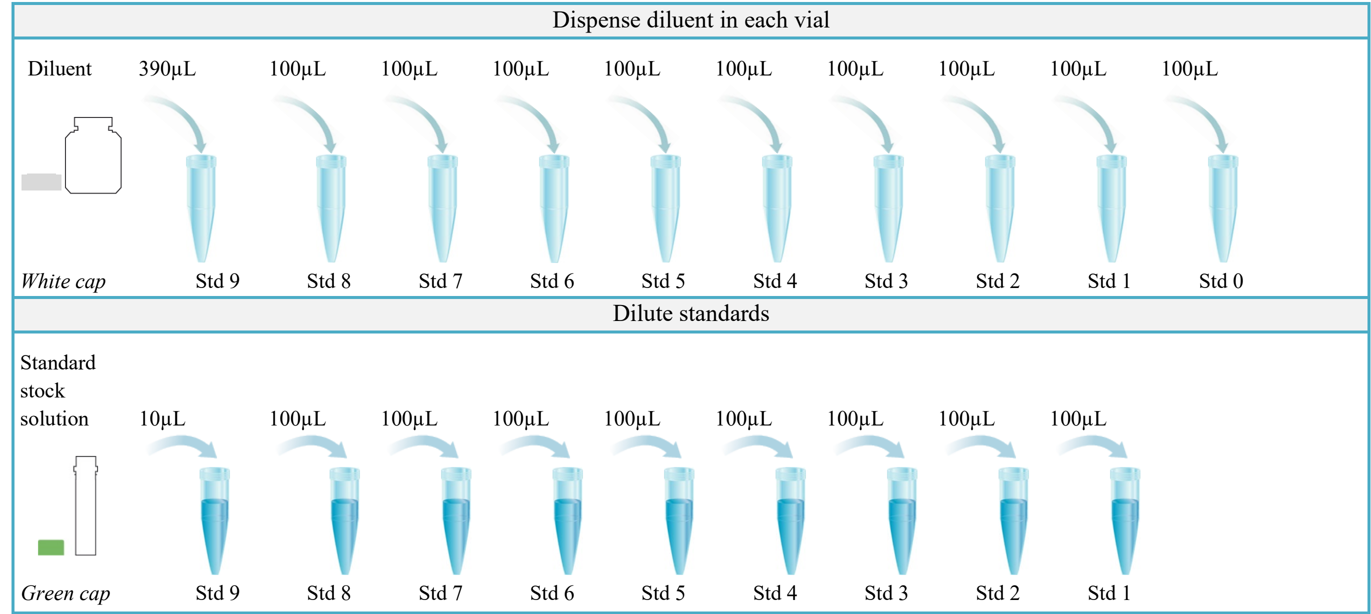
Determine how many standard levels and replicates to be tested. Each well requires 10µL of standard.

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	100	10µL of Std stock solution + 390µL diluent
Std 8	50	100µl Std 9 + 100µl diluent
Std 7	25	100µl Std 8 + 100µl diluent
Std 6	12.5	100µl Std 7 + 100µl diluent
Std 5	6.25	100µl Std 6 + 100µl diluent
Std 4	3.1	100µl Std 5 + 100µl diluent
Std 3	1.6	100µl Std 4 + 100µl diluent
Std 2	0.8	100µl Std 3 + 100µl diluent
Std 1	0.4	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 40-fold with diluent. This yields the high standard (Std 9 : 100 mg/mL) for the top of the curve. In practice:
  - e.g. take 10µL of the standard stock solution and add it to 390µL 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: 50, 25, 12.5, 6.25, 3.1, 1.6, 0.8, 0.4 ng/mL. This will create 9 standards for the analyte.
  - Std 0 (negative control) is diluent alone.



4. ASSAY PROTOCOL

Dispense the reagents in the following order:

10µL Standard or Sample → 5µL Anti-(h)IgG Kappa-d2 antibody → 5µL Anti-(h)Fc-Eu^{3+}-Cryptate antibody

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 more information about compatible reader at www.cisbio.com/readers

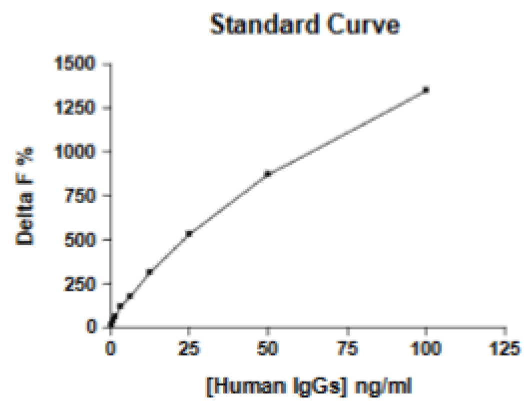
	Assay controls			
	Negative control	Cryptate control	Buffer control	Sample / Std
	Used to calculate the delta F%	*used to check the Cryptate signal at 620 nm*	*used to check background fluorescence*	
Sample / Std	-	-	-	10µL
Diluent	10µL	10µL	10µL	-
Anti-(h) IgG Kappa-d2 antibody	5µL	-	-	5µL
Anti-(h)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

5. 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	948	2.0	0
Std 1 – 0.4	1089	2.4	15
Std 2 – 0.8	1298	2.8	37
Std 3 – 1.6	1520	1.4	60
Std 4 – 3.1	2066	2.9	118
Std 5 – 6.25	2608	1.5	175
Std 6 – 12.5	3921	1.0	314
Std 7 - 25	5993	1.0	532
Std 8 - 50	9220	1.2	872
Std 9 - 100	13750	0.7	1350



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		

6. ASSAY CHARACTERISTICS

7.1. Cross-reactivity

	Cross-reactivity %
Human Kappa	100
Human Lambda	0
Mouse Kappa	0
Human IgM (Kappa)	<1

7.2. Detection limit

Human Kappa (IgG1) = 0.8 ng/mL

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