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Mouse IGF-1 kit
500 tests

For in vitro research use only

Storage temperature : -20°C or below

Packaging details :

	384-well low volume plate (20 µL)
63ADK090PEG	500 tests

Product information:

Document reference : 63ADK090PEG - rev01-Aug.2017

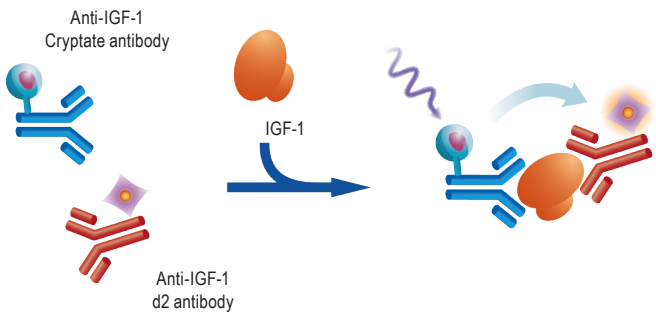
1. ASSAY DESCRIPTION AND INTENDED USE

This assay is intended for the quantitative determination of Mouse IGF-1 (IGF-1) using HTRF® technology.

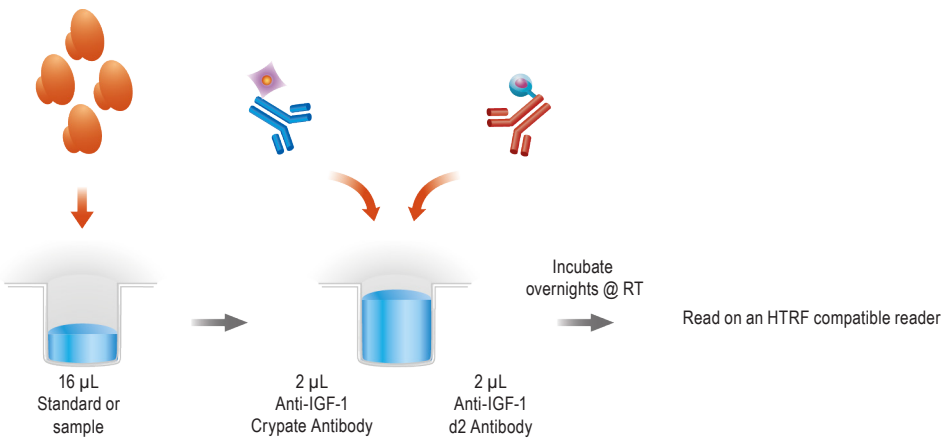
As shown in the diagram to the right, IGF-1 is detected in a sandwich assay format using two different specific antibodies, one labeled with Eu^{3+}-Cryptate (donor) and the second with d2 (acceptor).

When these 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 antigen present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen–antibody complexes formed and therefore to the IGF-1 concentration.



2. PROTOCOL AT GLANCE



3. HTRF REAGENTS

	IGF-1 Standard	Anti -IGF-1 d2 antibody	Anti -IGF-1 Eu^{3+}-Cryptate-antibody	Diluent	Detection buffer
					
Stock solution	10 µL/vial 200 µg/mL	20 µL/vial	20 µL/vial	20 mL/vial	2 mL/vial
Storage	-20°C or below	-20°C or below	-20°C or below	4°C to -60°C*	4°C to -20°C*
nb of vial	1	1	1	1	1
Ref# (when available sperately)	-	-	-	62DL1DDD	-

* Detection buffer is shipped frozen, but can be stored at 2-8°C.

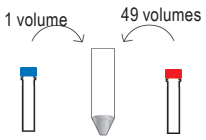
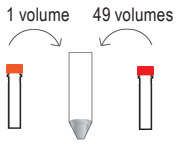
4. REAGENT PREPARATION

HTRF® reagent concentrations have been set for optimal assay performance. Any dilution or improper use of the d2 and Cryptate-antibodies will impair the quality of the assay.
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).
Antibodies may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended that you dispense remaining stock solutions of standard and antibodies into disposable plastic vials for storage at –20°C or below.
Please note, working solution preparation may differ between the 500 and the 10,000 data point size kits.

- Thaw all reagents at room temperature, allow them to warm up.
- Prepare the working solutions from stock solutions by following the instructions below.

4.1. Preparation of antibody working solutions

Determine the amount of antibody needed for the experiment. Each well requires 2 µL of each antibody.

Anti-IGF-1-d2 antibody	Anti-IGF-1-Eu^{3+}-Cryptate antibody
	
Dilute 50-fold the stock solution of anti-mouse IGF-1-d2 antibody with detection buffer : e.g. take 20 µL of d2-antibody stock solution and add it to 980 µL of detection buffer .	Dilute 50-fold the stock solution of anti-mouse IGF-1-cryptate antibody with detection buffer : e.g. take 20 µL of cryptate-antibody stock solution and add it to 980 µL of detection buffer .

4.2. Standard curve preparation

Determine how many samples and replicates will be tested.
Each well requires 16 µL of sample or standard. Standard curve can be prepared in the medium used for the preparation of the samples

Standards	Working concentration (pg/mL)	Preparation
Standard Stock Solution	200 000 000	See vial label
Intermediate Standard Solution # A	2 000 000	4 µL Standard Stock Solution + 396 µL diluent
Intermediate Standard Solution # B	20 000	4 µL Intermediate Standard Solution # A +396 µL diluent
Std 7	2 000	20 µL Intermediate Standard Solution # B + 180 µL diluent
Std 6	1 000	100 µL Std 7 + 100 µL diluent
Std 5	500	100 µL Std 6 + 100 µL diluent
Std 4	250	100 µL Std 5 + 100 µL diluent
Std 3	125	100 µL Std 4 + 100 µL diluent
Std 2	62.5	100 µL Std 3 + 100 µL diluent
Std 1	31.25	100 µL Std 2 + 100 µL diluent
Std 0	0	100 µL diluent

A recommended standard dilution procedure is listed and illustrated below:

1. Dilute the standard stock solution 100-fold with diluent; this yields the Intermediate Standard Solution # A (2,000,000 pg/mL). e.g: take 4 µL of standard stock solution and add it to 396 µL of diluent. Mix gently

2. Dilute this Intermediate Standard Solution # A 100-fold with diluent; this yields the Intermediate Standard Solution # B (20,000 pg/mL). e.g: take 4 µL of standard stock solution and add it to 396 µL of diluent. Mix gently

3. Dilute this Intermediate Standard Solution # B 10-fold with diluent to prepare high standard (Std 7: 2,000 pg/mL) for the top of the curve: e.g: take 20 µL of Intermediate Standard Solution # B and add it to 180 µL of diluent. Mix gently.

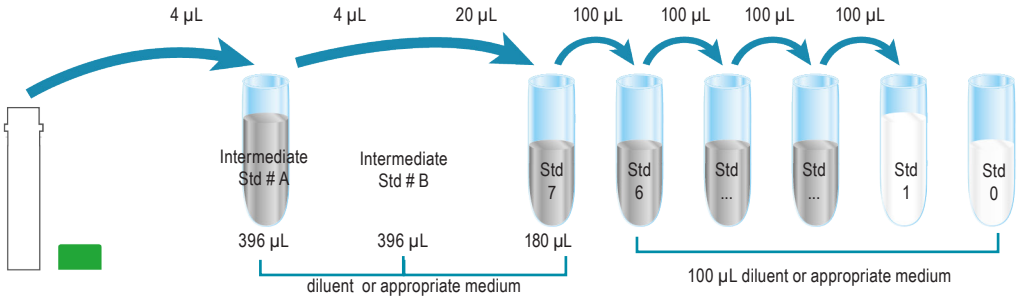
4. Use the high standard (Std 7: 2,000 pg/mL) to prepare the standard curve using 1/2 serial dilutions as follows:

   • Dispense 100 µL of diluent in each vial from Std 6 to Std 0.

   • Add 100 µL of standard to 100 µL of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: 1 000 - 500 - 250 - 125 - 62.5 - 31.25 pg/mL.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent alone.

The standard dilution procedure is listed and illustrated below:



5. ASSAY PROTOCOL

Dispense the reagents in the following order:

16 µL standard or sample → 2 µL anti-IGF-1-d2 antibody* → 2 µL anti-IGF-1-Eu^{3+}cryptate antibody*

*The two working antibody solutions must be prepared in individual vials and can be mixed prior to dispense. Add 1 volumee of anti-IGF-1-d2 antibody to 1 volume of anti-IGF-1-Eu^{3+} crypate antibody, and dispense 4 µL of the pre-mix anti-Mouse IGF-1 antibodies.

→ Cover the plate with a plate sealer.

→ Incubate overnights @ RT.

→ Remove the plate sealer and,

→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF® reader, using the appropriate set-up for Eu^{3+}

Use white plate only

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

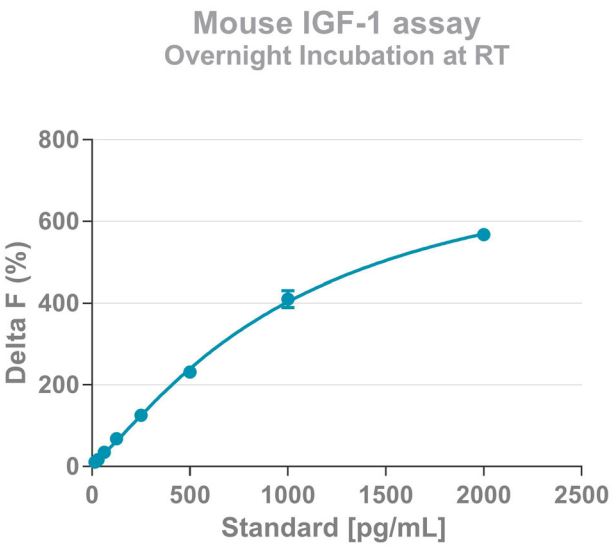
	Assay controls		
	Negative control	Cryptate control	Sample / Std
	Used to calculate the delta F %	*Used to check the Cryptate signal at 620nm*	
Sample / Std	-	-	16 µL
Diluent	16 µL	16 µL	-
Anti - IGF-1 d2 antibody	2 µL	-	2 µL
Anti - IGF-1 Eu^{3+}-Cryptate antibody	2 µL	2 µL	2 µL
Detection buffer	-	2 µL	-

6. DATA REDUCTION

This data must not be substituted for that obtained in the laboratory and should be considered only as an example (obtained on a PHERAstar FS using a 384-low volume white plate). Results may vary from one HTRF® compatible reader to another.

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

	[IGF-1] (pg/mL)	Ratio (1)	CV % (2)	Delta F % (3)
Std 0	0	715	1%	0%
Std 1	31.25	834	1%	17%
Std 2	62.5	966	4%	35%
Std 3	125	1202	1%	68%
Std 4	250	1609	3%	125%
Std 5	500	2367	3%	231%
Std 6	1000	3648	4%	410%
Std 7	2000	4775	2%	568%



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)	$\frac{\text{Ratio standard or sample} - \text{Ratio Negative control}}{\text{Ratio Negative control}} \times 100$	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: http://www.cisbio.com/data-reduction		

To obtain additional information or support, please contact the HTRF technical support team at: htrfservices@cisbio.com

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INTERACTION IS EVERYTHING