

Japan Office

USA Office

For in vitro research use only
 Storage temperature : 2-8°C

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HTRF® package insert

Document reference : 62IL1PEC rev05 (July 2008)

Packaging details :

	384-well low volume plate (20 µL)
62IL1PEC	20,000 tests

1. Assay description and intended use

This kit is intended for the quantitative determination of human Interleukin 1 beta.

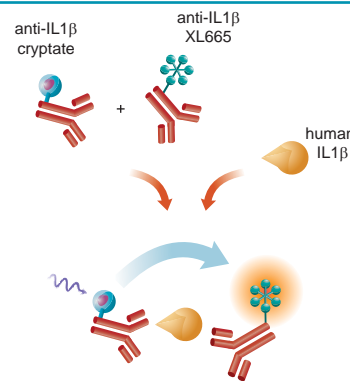
Its principle is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown below, IL1β is detected by an anti-IL1β MAb labeled with XL665, the second MAb is labeled with Cryptate. These mouse MABs recognize distinct epitopes of human IL1β. They do not crossreact with IL1β from other species.

Specific signal (i.e. energy transfer) is proportional to the concentration of IL1β in the sample or calibrator.

The human IL1β assay can be run under two different protocols:

1. The “supernatant” protocol (see §3), a standard IL1β assessment in cell supernatant : i) cells are stimulated in a regular cell culture plate, and ii) cell supernatant is then transferred to the assay plate.
2. The “cell-based” protocol (see §4) is carried out in a single plate and allows the quantification of IL1β directly on stimulated cells, without any transfer steps.

CAUTION ! Reagent reconstitution differs between the two protocols. Make sure to reconstitute the conjugates according to the chosen protocol's specifications.

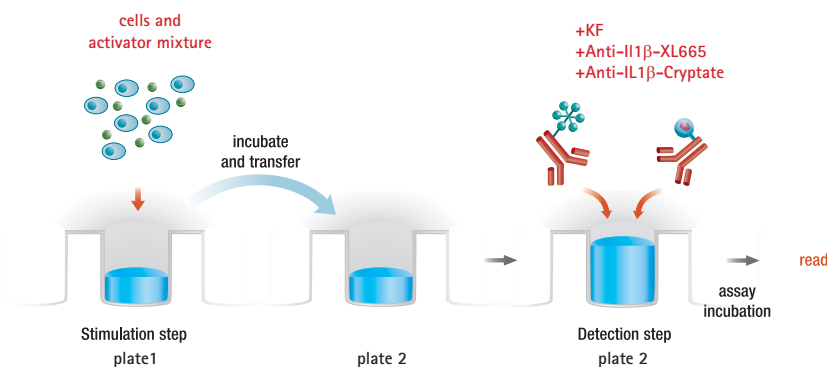


2. Background

Interleukin-1 was first described as a polypeptide derived from mononuclear phagocytes that costimulates T cells activation. It is now well established that IL-1 is merely a mediator of the host inflammatory response in natural immunity. The major source of IL-1 is the activated macrophage when triggered by LPS or other macrophage-derived cytokines such as TNFα and IL-1 itself. IL-1 is secreted under two distinct molecular forms, IL-1α and IL-1β, stemming from two different genes. Both polypeptides are produced as 33 kDa precursor molecules which have to be cleaved by ICE/caspase 1 to give a mature 17 kDa molecule. Both IL-1α and IL-1β exert their effect on a broad range of targets and they mediate similar biological activities which include production of acute phase proteins by hepatocytes, fever, induction of PGE2 synthesis by synovial cells and release of collagenase. Despite a sequence homology of only 20% IL-1α and IL-1β share the same surface receptors. IL-1α has an isoelectric point of 5 and is mostly cell membrane associated, whereas IL-1β with an isoelectric point of 7 is secreted.

3. Supernatant assay protocol

The supernatant assay protocol must be run in two distinct microplates : i) a culture plate for cell stimulation and ii) an assay plate in which cytokine detection is carried out.



3.1. Supplied reagents and reconstitution

Allow the reagents to warm up at room temperature for at least 30 mins and reconstitute all vials as indicated below.

Supplied reagents	Reagent reconstitution (stock solutions) *	Working solutions
anti IL1 β - Cryptate** 1 vial, lyophilized	Reconstitute each vial with 5 mL of distilled water. Mix gently.	⇒ For each vial dilute 1 volume of reconstituted reagent in 19 volumes of reconstitution buffer (e.g. for 10,000 tests: 2.5 mL of reconstituted reagent + 47.5 mL of reconstitution buffer).
anti IL1 β - XL665 1 vial, lyophilized		
IL1 β calibrator. Concentrated recombinant IL1 β . See label indications for concentration after reconstitution.	See label indications for reconstitution volume. Mix gently.	⇒ See calibration curve preparation for further dilution
IL1 β control. Concentrated recombinant IL1 β . See label indications for concentration after reconstitution.		
Reconstitution buffer (200 mL) 50 mM Phosphate buffer, pH 7.0, 0.8M KF, 0.2 % BSA	<p><i>Note: Supplementary IL1β maximum calibrator (ref 62IL1CDA) and diluent (ref 62DL1DDD) can be obtained separately on request.</i></p>	
Diluent (20 mL) 50 mM Phosphate buffer, pH 7.0, 0.2 % BSA, 0.02 % NaN ₃ , preservatives		

* All lyophilized reagents must be reconstituted with distilled water.

** The Cryptate conjugate concentration was optimized for a maximum assay sensitivity and to ensure an average counting of 40,000 cps at 620 nm (384-well low volume format), using the reference PHERAstar Plus reader (BMG LABTECH).

Precaution : HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the XL665 and Cryptate-conjugates will impair the assay's quality.

3.2. Reagent stability

All reagents should be stored at 2-8°C until reconstituted. Under proper storage conditions, they are stable until the expiry date indicated on the labels. Reconstituted reagents are stable for 48 hours at 4°C. They can be refrozen (at -80°C) and thawed at least one more time.

3.3. Calibration curve preparation

Follow the dilution sequence shown in the table below to constitute the calibration curve. Dilution must be carried out with the diluent (or with freshly made PO₄ 50 mM, BSA 0.1% pH7).

Calibrator	Working concentration in pg/mL	Preparation
Cal 7	2000	100 μ L reconstituted reagent + 400 μ L diluent
Cal 6	1000	250 μ L Cal 7 + 250 μ L diluent
Cal 5	500	250 μ L Cal 6 + 250 μ L diluent
Cal 4	200	200 μ L Cal 5 + 300 μ L diluent
Cal 3	100	250 μ L Cal 4 + 250 μ L diluent
Cal 2	50	250 μ L Cal 3 + 250 μ L diluent
Cal 1	20	200 μ L Cal 2 + 300 μ L diluent

* [IL1 β] is indicated on the label of the calibrator vial.

3.4. Sample preparation

Dilute all samples to be assayed with the diluent (or with freshly made PO₄ 50 mM, BSA 0.1% pH7). Consecutive dilutions should be made within the 0 to 2000 pg/mL range (working solution).

3.5. Assay protocol for 384-well low volume (20 μ L)

⇒ Dispense the reagents in the following order :

- 10 μ L standard or sample *
- 5 μ L anti-IL1 β Cryptate
- 5 μ L anti-IL1 β XL665

* For negative control, replace the first reagent by 10 μ L of diluent.

⇒ Cover the plate with a plate sealer and let the incubation take place at room temperature for 2 hours.

⇒ Read on a compatible HTRF® reader (more information about compatible reader at htrf-assays.com/readers).

3.6. Data reduction

This data should not be substituted for that obtained in the laboratory.

	A (665nm)	B (620nm)	Ratio (1)	Mean Ratio (2)	CV % (3)	Delta F % (4)
Negative control	1836 2036	47242 48804	397 409	403	2.1	
[IL1β] pg/mL						
20	1885 2194	44626 48771	422 450	436	4.5	8
50	2105 2258	46600 47175	452 479	465	4.1	15
100	2451 2514	45599 46371	538 542	540	0.6	34
200	3227 2834	47227 41716	683 679	681	0.4	69
500	5250 5142	48467 47155	1083 1090	1087	0.5	170
1000	8057 8266	48326 47942	1667 1724	1696	2.4	321
2000	14442 14863	48385 49131	2985 3025	3005	0.9	646
IL1β control	5012 5353	47957 49967	1045 1071	1058	1.8	163

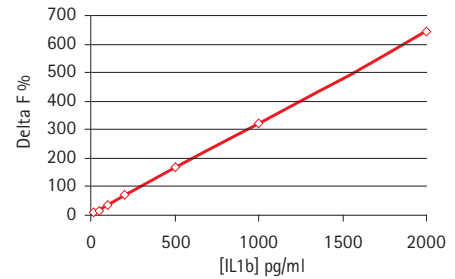
$$1. \text{Ratio} = \frac{A_{665\text{nm}}}{B_{620\text{nm}}} \times 10^4$$

$$2. \text{Mean ratio} = \frac{\sum \text{ratios}}{2}$$

$$3. \text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$$

$$4. \text{Delta F} = \frac{\text{Calibrator or sample Ratio} - \text{Ratio}_{\text{neg}}}{\text{Ratio}_{\text{neg}}} \times 100$$

(Ratio_{neg} = negative control)



3.7. Assay characteristics

Detection limit (dose of mean zero + 2 SD)	Hook effect	Linear range
< 15 pg/mL	> 100,000 pg/mL	up to 10,000 pg/mL

This immunoassay is calibrated against the NIBSC/WHO standard IL1β 86/680. To convert sample values obtained with HTRF® IL1β kit to equivalent NIBSC units, use the equation below :

$$\text{NIBSC/WHO (86/680) equivalent value (U/mL)} = 0.1 \times \text{IL1}\beta \text{ value (pg/mL)}$$

3.8. Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 20,000 tests using a 384- well low volume plate in 20 µL final assay volume (HTRF® packaged basis).

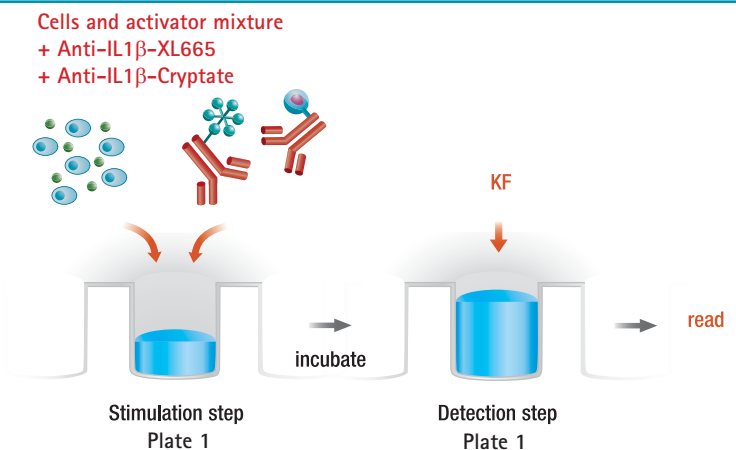
To move to other plate formats (96 half-well or 1536-well) and final volumes (100 µL to less than 10 µL), the volume of each assay component is simply proportionally adjusted in order to maintain the reagent concentrations as for the 20 µL final assay volume. For instance, in the case of the 1536-well format in 10 µL final volume, half as much material per well is used, thereby allowing 40,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

Assay components	Volume proportion	Assay format		
		1536-well (10 µL)	384-well low volume (20 µL)	96 half-well (100 µL)
Sample	2 volumes	5 µL	10 µL	50 µL
XL665 conjugate	1 volume	2.5 µL	5 µL	25 µL
Cryptate conjugate	1 volume	2.5 µL	5 µL	25 µL
	Bulk size	40,000 tests	20,000 tests	4,000 tests

Plate references : 96 half-well plate (Costar # 3694 or equivalent), 384-well low volume plate (Greiner # 784076), 1536-well (Greiner # 782086).

4. Cell-based assay protocol

In this protocol, reagent reconstitution and distribution have been modified in order to allow the direct measurement of IL1β on stimulated cells. This protocol is particularly well adapted to secondary screening.



4.1. Reagent reconstitution

Anti-IL1 β - Cryptate	⇒	Reconstitute each vial with 5 mL of distilled water . Mix gently. Dilute 1 volume of reconstituted reagent in 7 volumes of culture medium (i.e. for 10,000 tests : 2.5 mL of reconstituted reagent + 17.5 mL of culture medium).
Anti-IL1 β - XL665		
IL1 β calibrator	⇒	<ul style="list-style-type: none"> • Calculate the reconstitution volume for each vial by dividing the reconstitution volume indicated on the labels of the vials by 1.67 (i.e. if 500 μL is printed on the calibrator label, the reconstitution volume will be 299.4 μL). • Use culture medium NOT WATER to reconstitute both calibrator and control.
IL1 β control		
Reconstitution buffer 50 mM Phosphate buffer, pH 7.0, 0.8M KF	⇒	Ready to use : in the cell-based assay, the reconstitution buffer is not used for conjugate reconstitution. It is added as KF buffer at the end of the incubation to allow HTRF [®] readout.

4.2. Reagent storage and stability Refer to § 3.2.

4.3. Calibration curve preparation

Reconstitute the maximum calibrator with the culture medium volume indicated on the label, divided by 1.67. Follow the same dilution sequence as shown in the table in § 3.3., **replacing the diluent by culture medium**.

4.4. Sample preparation

Cell density optimization is a key step in the IL1 β cell-based assay. Typically, the level of IL1 β produced by cells must fall within the 100-2000 pg/mL range. Optimization consists of testing a broad range of cell concentrations (e.g. between 100 and 20,000 cells per well) in the presence or the absence of a direct activator of IL1 β production, e.g. LPS at 10 μ g/mL. In practice, resuspend the cells in the culture medium supplemented with the activator(s) or inhibitor(s) so that the desired number of cells will be dispensed under 6 μ L.

4.5. Cell-based assay protocol for 384-well low volume (20 μ L)

Dispense the reagents in the following order:

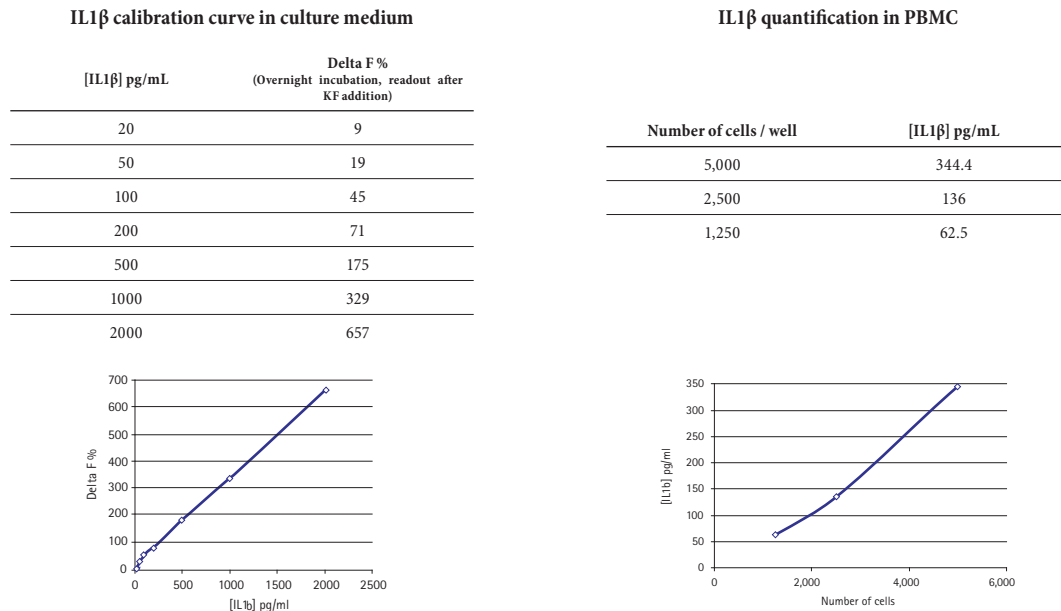
- 6 μ L standard or cell suspension
- 2 μ L anti-IL1 β Cryptate
- 2 μ L anti-IL1 β XL665

* For negative control, replace the first reagent by 6 μ L of culture medium.

- Incubate the plate for 18 to 24 hours at 37°C in a CO₂ incubator with a fully humidified atmosphere.
- Add 10 μ L of reconstitution buffer.
- Read on a compatible HTRF[®] reader (more information about compatible reader at htrf-assays.com/readers)

4.6. Case study : IL1 β quantification in peripheral blood mononucleated cells (PBMC)

- PBMC were separated on a ficoll density gradient ($d=1.077$) (Sigma). Cells were washed twice and viability was evaluated by trypan blue dye exclusion. Cells were then resuspended in RPMI1640 culture medium complemented with 10% heat inactivated calf serum, and stimulated with 10 μ g/mL of LPS.
- A graduated number of cells per well were distributed in 6 μ L of culture medium including the LPS activator, according to the cell-based assay protocol described above.



The data shows that the co-incubation of the stimulated cells with the labeled antibody pair does not alter HTRF[®] readout or assay performances. IL1 β quantification is proportional to the number of cells per well. In this example, the cell density selected for a secondary screen to determine inhibitors of IL1 β production would be 5,000 cells/well. In all instances, cell density should be carefully optimized, as the secretion of IL1 β may vary between cell types.