MANUAL

Technology: HTRF™ Cytokine

HTRF Human pro IL1 beta Detection Kit

Part number	62HPIL1BPEG	62HPIL1BPEH
Test size	500 tests	10,000 tests

Storage: ≤ -16°C

Version: 01 **Date:** January 2026

ASSAY PRINCIPLE

This kit is designed for the simple and rapid quantification of the human pro IL1 beta receptor in cellular lysates. It provides a fast and convenient alternative to traditional ELISA methods.

The detection principle relies on HTRF™ technology (Homogeneous Time-Resolved Fluorescence). As illustrated in Figure 1, human pro IL1 beta is measured using a sandwich assay format by using anti- pro IL1 beta antibodies: one labeled with Europium cryptate (donor) and the other 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 fluorescence at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed an therefore to the pro IL1 beta concentration.

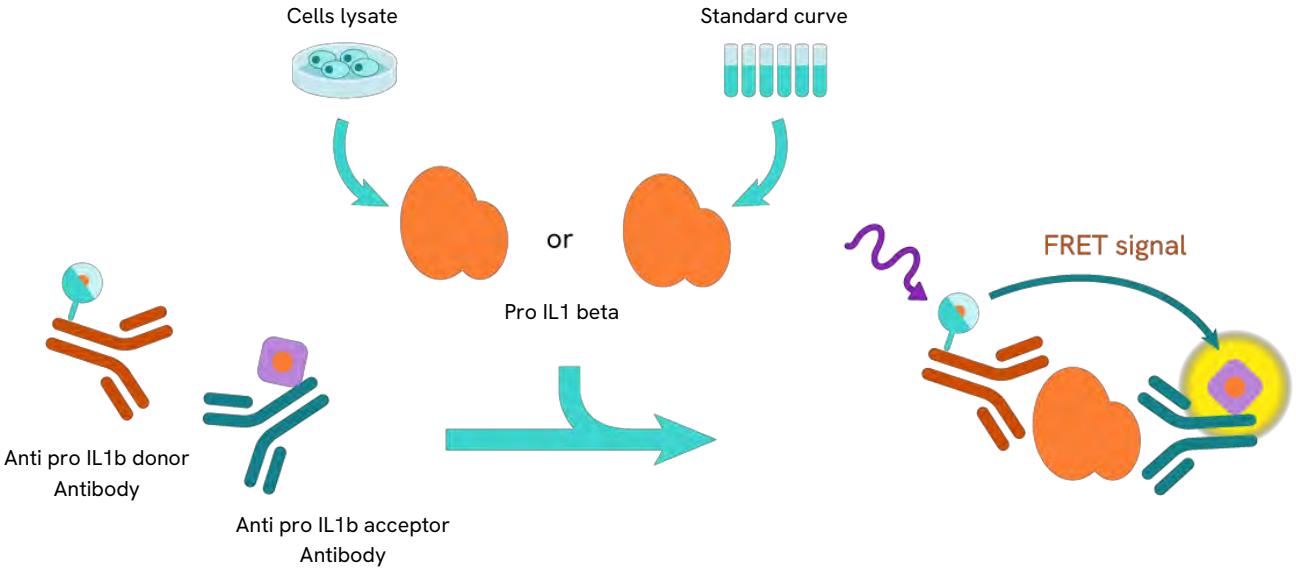
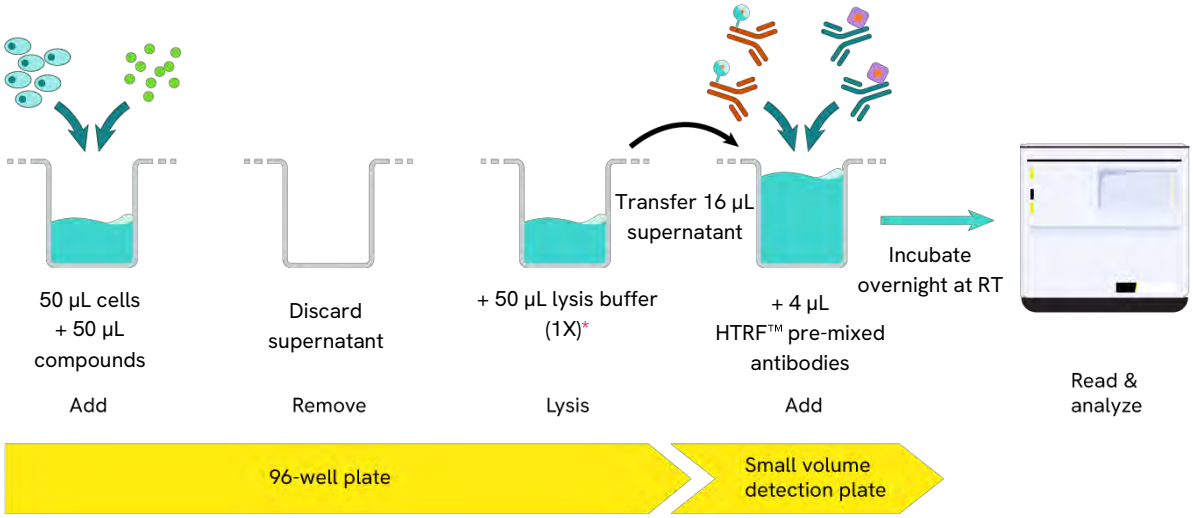


Figure 1: Principle of HTRF pro IL1 beta sandwich assay

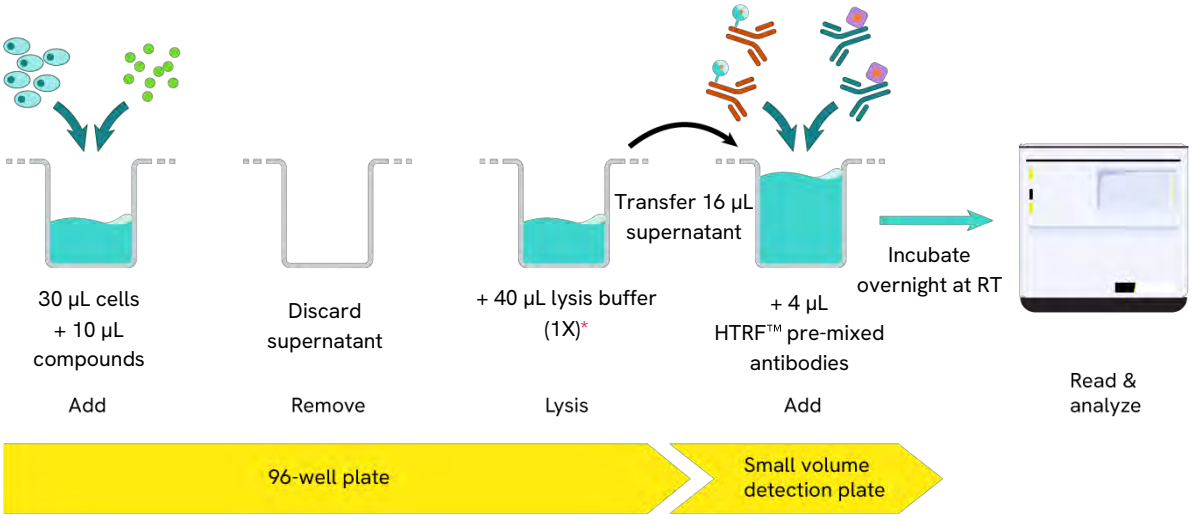
PROTOCOL AT A GLANCE

Two-plate assay protocol for adherent cells



Make sure to use the set-up for Eu Cryptate.

Two-plate assay protocol for suspension cells



MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*			10,000 TESTS*		
Pro IL1 beta Standard Lyophilized		green cap	2 vials		green cap	2 vials
Pro IL1 beta Eu Cryptate Antibody Frozen 20X		orange cap	1 vial 50 µL		red cap	1 vial 1 mL
Pro IL1 beta d2 Antibody Frozen 20X		blue cap	1 vial 50 µL		purple cap	1 vial 1 mL
Lysis buffer #1 (stock solution 4X)		transparent cap	4 vials 2 mL		white cap	1 vial 130 mL
Detection Buffer** #3 Ready-to-use		red cap	2 vials 1.5 mL		red cap	1 vial 50 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

Purchase separately

- HTRF™-Certified Reader. Make sure the setup for Eu Cryptate is used. For a list of HTRF-compatible readers and set-up recommendations, please visit our website.
- Small volume (SV) detection microplates. Use white plate only. For more information about microplate recommendations, please visit our website.

STORAGE AND STABILITY

Kit

- Store the kit at -16°C or below.
- Under proper storage conditions, reagents are stable until the expiry date indicated on the label.
- Lysis buffer and detection buffer are shipped frozen but can be stored at 2-8°C in your premises.

Reagents

- If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
- Volume of pro IL1 beta standard aliquots should not be under 10 µL and stored at -60°C or below.

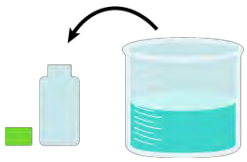
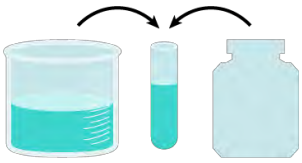
REAGENT PREPARATION

Before you begin

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use.
- Before use, allow lysis buffer and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Pro IL1 beta standards (for standard curve) must be prepared in the same lysis buffer as the samples.

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

To prepare reagent stock solutions

500 TESTS			10,000 TESTS
Anti-pro IL1 beta Eu Cryptate antibody			
Thaw the pro IL1 beta Eu Cryptate antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.			Thaw the pro IL1 beta Eu Cryptate antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
Anti-pro IL1 beta d2 antibody			
Thaw the pro IL1 beta d2 antibody. Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.			Thaw the pro IL1 beta d2 antibody . Mix gently. This 20X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
pro IL1 beta Standard			
Reconstitute the pro IL 1 beta standard with distilled water. Volume of reconstitution is indicated on the vial label. The reconstituted standard solution can be frozen and stored at -60°C or below.			Reconstitute the pro IL 1 beta standard with distilled water. Volume of reconstitution is indicated on the vial label. The reconstituted standard solution can be frozen and stored at -60°C or below.
Lysis buffer			
Dilute 4-fold the 4 X Lysis buffer with distilled water: homogenize the 4 X Lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 1 mL of lysis buffer + 3 mL of distilled water). Mix gently after dilution. This 1X lysis buffer can be frozen and stored at -16°C or below.			Dilute 4-fold the 4 X Lysis buffer with distilled water: homogenize the 4 X Lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 10 mL of lysis buffer + 30 mL of distilled water). Mix gently after dilution. This 1X lysis buffer can be frozen and stored at -16°C or below.
Detection buffer			

The Detection buffer is ready-to-use.

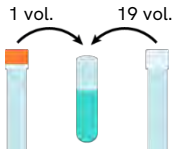
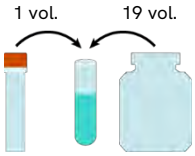
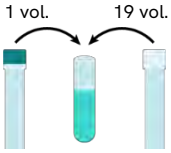
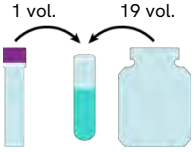
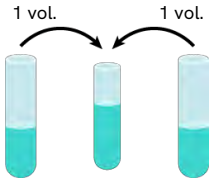
To prepare antibody working solutions

Each well requires 2 µL of pro IL1 beta -Eu Cryptate Antibody and 2 µL of pro IL1 beta d2 Antibody.

Prepare the two antibody solutions in separate vials.

To prepare working standards solutions

- Each well requires 16 µL of standard.

500 TESTS		10,000 TESTS	
Anti-pro IL1 beta Eu Cryptate antibody			
Dilute 20-fold the 20X stock solution (thawed reagent) of pro IL1 beta Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 19 volumes of detection buffer (e.g. 10 µL of Eu Cryptate antibody stock solution + 190 µL of detection buffer).			Dilute 20-fold the 20X stock solution (thawed reagent) of pro IL1 beta Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 19 volumes of detection buffer (e.g.10 µL of Eu Cryptate antibody stock solution + 190 µL of detection buffer).
Anti-pro IL1 beta d2 antibody			
Dilute 20-fold the 20X stock solution (thawed reagent) of pro IL1 beta d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 10 µL of Eu Cryptate antibody stock solution + 190 µL of detection buffer).			Dilute 20-fold the 20X stock solution (thawed reagent) of pro IL1 beta d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 10 µL of Eu Cryptate antibody stock solution + 190 µL of detection buffer).
Antibody Mix			
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).			It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 20 mL of d2 antibody + 20 mL of Cryptate antibody).

- Dilute the standard stock solution serially with LB#1 (1X)
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

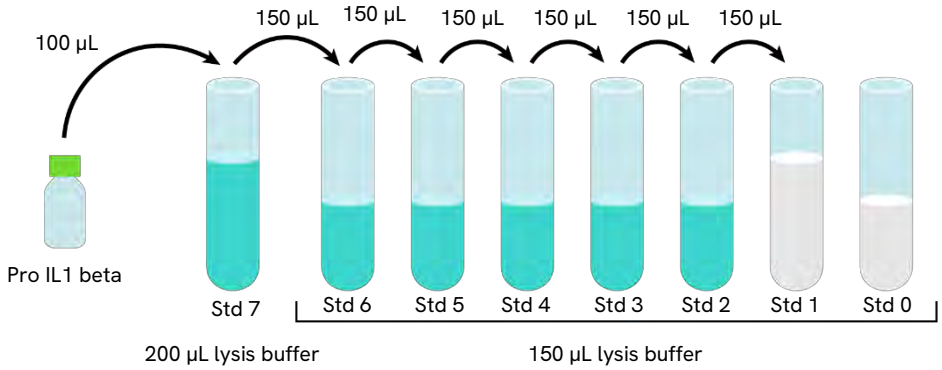
Dilute the standard stock solution 3-fold with LB#1 (1X); this yields the Standard Max solution (60,000 pg/mL)

Dilute the standard stock solution 3-fold with LB#1 (1X) to prepare high standard (Std 7): e.g. take 100 µL of standard stock solution and add it to 200 µL of LB#1 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using serial dilutions as follows:

- Dispense 150 µL of LB #1 (1X) in each vial from Std 6 to Std 0.
- Add 150 µL of standard to 150 µL of LB #1 (1X), mix gently and repeat the serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is LB #1 (1X)



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Reconstituted lyophilisate	60 000 pg/mL
Standard 7	100 µL Standard stock Solution + 200 µL lysis buffer 1X	20 000 pg/mL
Standard 6	150 µL standard 7 + 150 µL lysis buffer 1X	10 000 pg/mL
Standard 5	150 µL standard 6 + 150 µL lysis buffer 1X	5 000 pg/mL
Standard 4	150 µL standard 5 + 150 µL lysis buffer 1X	2 500 pg/mL
Standard 3	150 µL standard 4 + 150 µL lysis buffer 1X	1 250 pg/mL
Standard 2	150 µL standard 3 + 150 µL lysis buffer 1X	625 pg/mL
Standard 1	150 µL standard 2 + 150 µL lysis buffer 1X	312.5 pg/mL
Standard 0	150 µL lysis buffer 1X	0

To prepare samples

- Each well requires 16 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted in LB #1 (1X).
- To obtain additional information or support, please contact the HTRF technical support team.

ASSAY PROTOCOL

		STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 16 µL of each pro IL1 beta standard (Std 0 - Std 7) into each standard well	Dispense 16 µL of each sample into each sample well
Step 2		Add 2 µL of pro IL1 beta d2 antibody working solution to all wells	
Step 3		Add 2 µL of pro IL1 beta Eu Cryptate antibody working solution to all wells.	
Step 4		Seal the plate and incubate overnight at RT	
Step 5		Remove the plate sealer and read on an HTRF™ compatible reader	

	1	2	3	4	5	6
A	16 µL Std 0 (Negative control) 2 µL pro IL1 beta-d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well A1	Repeat Well A1	16 µL sample 1 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well B1	Repeat Well B1	16 µL sample 2 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well C1	Repeat Well C1	16 µL sample 3 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well C4	Repeat Well C4
D	16 µL Std 3 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well D1	Repeat Well D1	16 µL sample... 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well D4	Repeat Well D4
E	16 µL Std 4 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well E1	Repeat Well E1	16 µL sample... 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well E4	Repeat Well E4
F	16 µL Std 5 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well F1	Repeat Well F1	16 µL sample... 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well F4	Repeat Well F4
G	16 µL Std 6 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well G1	Repeat Well G1	16 µL sample... 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well G4	Repeat Well G4
H	16 µL Std 7 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well H1	Repeat Well H1	16 µL sample... 2 µL pro IL1 beta -d2 2 µL pro IL1 beta -Eu Cryptate	Repeat Well H4	Repeat Well H4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
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DATA REDUCTION & INTERPRETATION

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.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit our website.

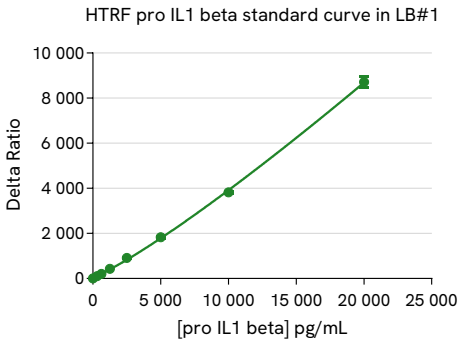
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF™ compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL with $1/Y^2$) model

		Ratio (1)	CV% (2)	Delta Ratio
Standard 0	Negative control	238	1%	0
Standard 1	312.5 pg/mL	346	4%	108
Standard 2	625 pg/mL	442	0%	204
Standard 3	1 250 pg/mL	672	1%	433
Standard 4	2 500 pg/mL	1149	1%	911
Standard 5	5 000 pg/mL	2066	3%	1828
Standard 6	10 000 pg/mL	4056	1%	3817
Standard 7	20 000 pg/mL	8950	3%	8712



REACH European regulations and compliance.

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

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