MANUAL

Technology: HTRF™ Cytokine

HTRF High Performance Human IL-6 Detection Kit

Part number:	62HIL6V2PET
Test size	96 tests

Storage: ≤ 20°C

Version: 01 **Date:** July 2024

ASSAY PRINCIPLE

Revvity's human IL-6 assay is only intended for the quantitative measurement of IL-6 in supernatant using HTRF™ technology. The assay is compatible with human samples, and is highly specific for IL-6.

IL-6 is detected in a sandwich assay format using 2 different specific antibodies, one labeled with Europium Cryptate (donor) and the second with d2 (acceptor).

The detection principle is based on HTRF™ technology. When the labelled antibodies bind to the same antigen, 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 (665 nm). The two antibodies bind to the IL-6 present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the IL-6 concentration. (Fig. 1).

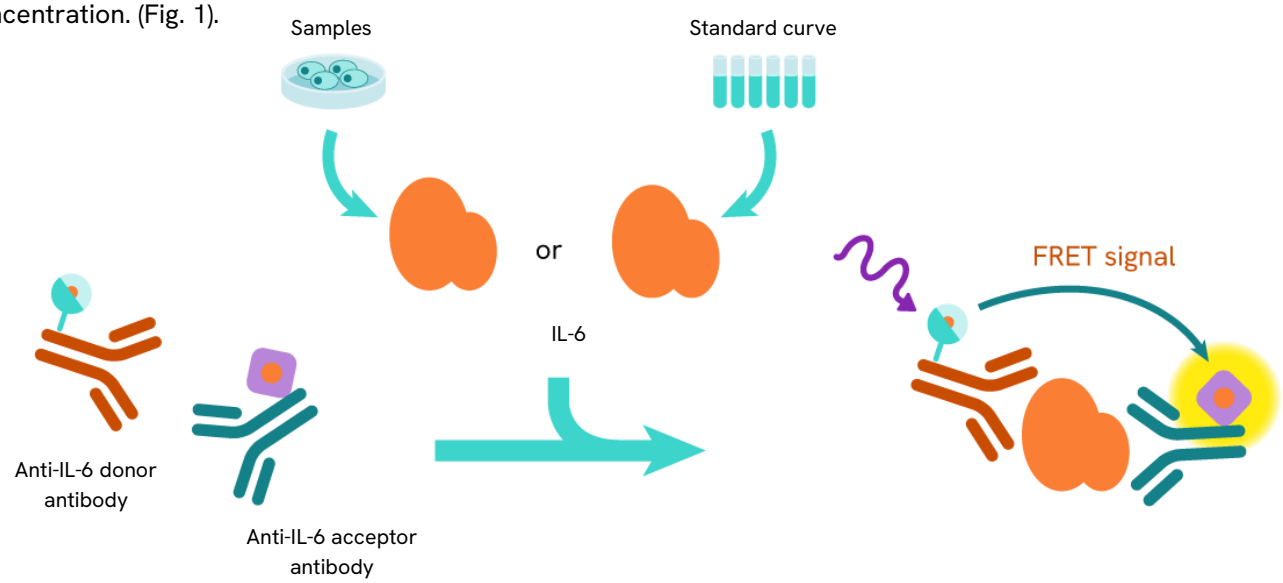
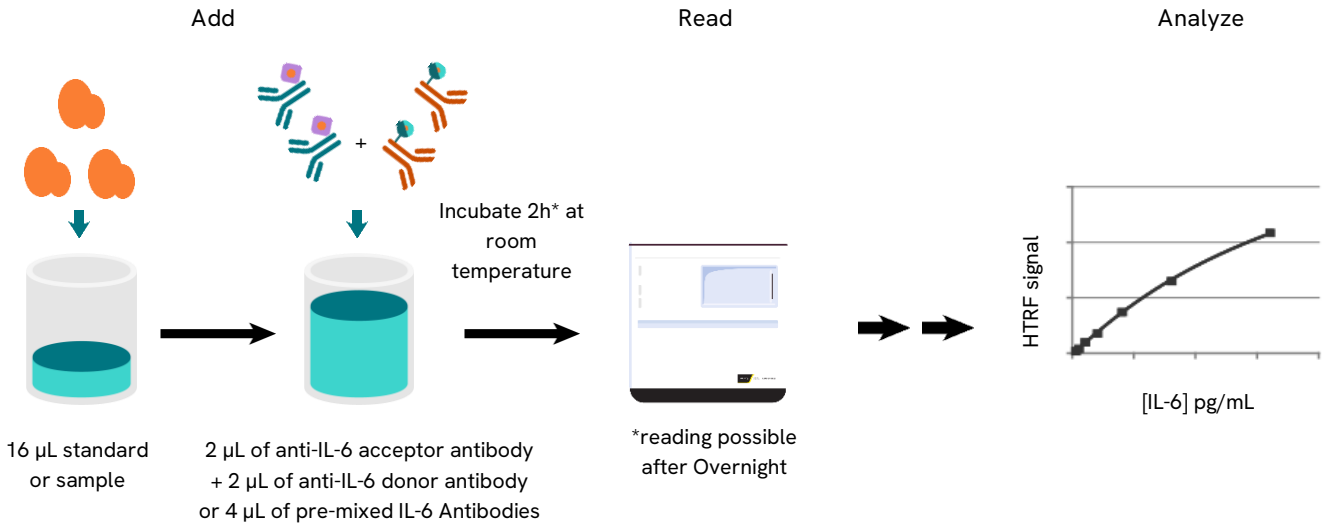


Figure 1: Principle of HTRF HP IL-6 sandwich assay

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS		96 TESTS	
IL-6 standard lyophilized		green cap	1 vial
IL-6 Eu cryptate antibody frozen 20X		orange cap	1 vial 10 µL
IL-6 d2 antibody frozen 20X		blue of cap	1 vial 10 µL
Diluent* #5 5X		white cap	1 vial 2 mL
Detection buffer #3 Ready-to-use**		rouge cap	1 vial 0.5 mL
Plate			1 plate HTRF 96-well low volume plate

* To prepare working standard solutions, culture medium can be an alternative the diluent.
** The Detection Buffer is used to prepare working solutions of acceptor and donor reagents

Purchase separately

- HTRF™-Certified Reader. For a list of HTRF-compatible readers and set-up recommendations, please visit our website.
- 96-well or 384-well small volume (SV) detection microplates. For more information about microplate recommendations, please visit our website.

STORAGE AND STABILITY

- Store the kit at -20°C or below.
- Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

Once reconstituted, standard stock solution may be frozen at ≤-60°C, and can be thawed only once.
Once thawed, antibody solutions can be frozen once.
To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at ≤-60°C.
Volume of standard and antibody aliquots should not be under 10 µL.
Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

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.
- Before use, allow all kit's reagents to warm up at room temperature then
    - homogeneize buffer and diluent with a vortex
    - centrifuge (NEVER vortex) the antibodies to gather all liquid at the bottom of the vial
- It is recommended to filter buffers before use.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.

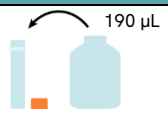
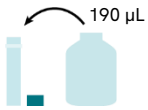
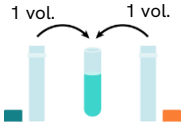
Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

To prepare reagent stock solutions

96 TESTS	
Anti-IL-6 Eu Cryptate antibody	
Thaw the IL-6 Eu Cryptate antibody. Centrifuge. This stock solution can be frozen and stored at ≤-60°C	
Anti-IL-6 d2 antibody	
Thaw the IL-6 d2 antibody. Centrifuge. This stock solution can be frozen and stored at ≤-60°C.	
IL-6 Standard	
Reconstitute the IL-6 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.	
Diluent	
Dilute 5-fold the 5 X diluent #5 with distilled water: homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water e.g. 1 mL of diluent + 4 mL of distilled water Mix gently after dilution.	

To prepare antibody working solutions

Each well requires 4 µL of pre-mixed IL-6 antibodies. Prepare the two antibody solutions in separate vials.

96 TESTS	
IL-6 Eu Cryptate antibody	
Dilute the stock solution (thawed reagent) of IL-6 Eu Cryptate-antibody with detection buffer #3: Add 190µL of detection buffer directly in the thawed Eu Cryptate-antibody stock solution.	
IL-6 d2 antibody	
Dilute the stock solution (thawed reagent) of IL-6 d2 antibody with detection buffer #3: Add 190µL of detection buffer directly in thawed d2 antibody stock solution.	
Antibody Mix	
Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents: e.g. 200 µL of d2 antibody + 200 µL of Eu Cryptate antibody	

To prepare working standards solutions

- Each well requires 16 µL of standard.
- Serially dilute the standard stock solution with diluent #5. or with the cell culture medium used to prepare your samples supplemented with BSA or 10% FCS.
- Due to the stability of the IL-6, it is mandatory to prepare the standard curve just before the assay.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent.
- In order to counteract any standard sticking we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

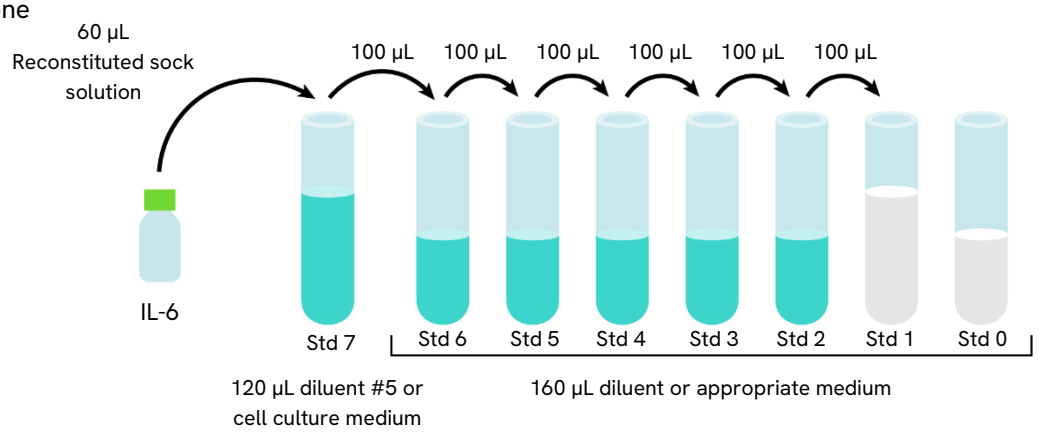
1. Reconstitute the standard vial with the volume indicated on the vial label using distilled water.
2. Prepare the following dilutions:

- Dilute the reconstituted standard stock solution 3-fold with diluent or with cell culture medium.

In practice: take 60 µL of stock solution and add it to 120 µL of diluent or cell culture medium. Mix gently. This yields the high standard (Std 7: 12 000 pg/mL) for the top of the curve.

- Use the high standard (Std 7) to prepare the standard curve using serial dilutions as follows:
  - Dispense 160 µL of diluent or cell culture medium into each vial from Std 6 to Std 0
  - Add 100 µL of standard to 160 µL of diluent or cell culture medium, 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 diluent or appropriate culture medium alone



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Reconstitute the vial following the indications given on the vial label	36 ng/mL
Standard 7	60 µL Standard stock Solution + 120 µL diluent	12 000 pg/mL
Standard 6	100 µL standard 7 + 160 µL diluent 1X	4615.4 pg/mL
Standard 5	100 µL standard 6 + 160 µL diluent 1X	1775.1 pg/mL
Standard 4	100 µL standard 5 + 160 µL diluent 1X	682.7 pg/mL
Standard 3	100 µL standard 4 + 160 µL diluent 1X	262.6 pg/mL
Standard 2	100 µL standard 3 + 160 µL diluent 1X	101 pg/mL
Standard 1	100 µL standard 2 + 160 µL diluent 1X	38.8 pg/mL
Standard 0	160 µL diluent 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 your appropriate sample medium.

ASSAY PROTOCOL

		STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 16 µL of each IL-6 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 IL-6 d2 antibody working solution to all wells	
Step 3		Add 2 µL of IL-6 Eu Cryptate antibody working solution to all wells.	
Step 4		Seal the plate and incubate 2h at RT *Reading possible after Overnight	
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 IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well A1	Repeat Well A1	16 µL sample 1 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well B1	Repeat Well B1	16 µL sample 2 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well C1	Repeat Well C1	16 µL sample 3 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	16 µL Std 3 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well D1	Repeat Well D1	16 µL sample... 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	16 µL Std 4 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well E1	Repeat Well E1	16 µL sample... 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	16 µL Std 5 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well F1	Repeat Well F1	16 µL sample... 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	16 µL Std 6 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well G1	Repeat Well G1	16 µL sample... 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well G4	Repeat Well G4
H	16 µL Std 7 2 µL IL-6-d2 2 µL IL-6-Eu Cryptate	Repeat Well H1	Repeat Well H1	16 µL sample... 2 µL IL-6-d2 2 µL IL-6-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
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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 delta ratio of the acceptor and donor emission signals for each individual well. The Standard 0 (Negative control) plays the role of an internal assay control.

$$\text{Delta Ratio} = \text{Ratio Standard or sample} - \text{Ratio Standard 0}$$

3) 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.

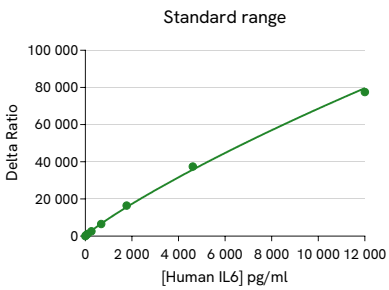
RESULTS

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)
Standard 0	**Negative control**	1117	8%
Standard 1	38.8 pg/mL	1777	5,1%
Standard 2	101 pg/mL	2409	4,4%
Standard 3	262.6 pg/mL	3831	0,5%
Standard 4	682.7 pg/mL	7714	3,2%
Standard 5	1775.1 pg/mL	17712	2,1%
Standard 6	4615.4 pg/mL	38652	1,2%
Standard 7	12 000 pg/mL	78740	2,6%



REACH European regulations and compliance.

This product and/or some of its components may 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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