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Hu IL-6 Chemiluminescence ELISA Kit

Catalog. no. KHC0069 **Quantity:** 96 tests

Pub. Part no. MAN0005182 **Rev 3.00**

Description

The IL-6 Chemiluminescence ELISA Kit is a solid-phase sandwich Enzyme Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of Human Interleukin-6 (Hu IL-6) in cell culture supernatants, serum, plasma or other body fluids. The assay will recognize both natural and recombinant Hu IL-6.

Human Interleukin-6 (IL-6) is a 184 A.A. polypeptide with potential O- and N-glycosylation sites and a significant homology with G-CSF. It is produced by various cells, including T- and B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma cells and several tumor cells. IL-6 regulates the growth and differentiation of various cell types with major activities on the immune system, hematopoiesis, and inflammation. These multiple actions are integrated within a complex cytokine network, where several cytokines induce (IL-1, TNF, PDGF, IFNs, etc.) or are induced by IL-6 and the final effects result from either synergistic or antagonistic activities between IL-6 and the other cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IFN-γ, GM-CSF, M-CSF, CSF, etc.). IL-6 induces final maturation of B-cells into antibody producing cells and is a potent growth factor for myeloma/plasmacytoma cells. It (co-) stimulates T-cell growth and cytotoxic T-cell differentiation. It promotes megakaryocyte development and synergizes with other cytokines to stimulate multipotent hematopoietic progenitors. IL-6 is also a major inducer of the acute phase reactions in response to inflammation or tissue injury. Along with IL-1 and TNF, it induces the synthesis of acute phase proteins (APP) by hepatocytes, each cytokine or combination of cytokines showing a preferential pattern of APP production. IL-6 also interacts with the neuroendocrine system, e.g., by inducing ACTH production. Thus, IL-6 is a pleiotropic cytokine with multiple endocrine, paracrine and possibly autocrine activities in various tissues.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2 to 8°C.

Components	Quantity
Hu IL-6 Antibody Coated Wells. 96 well plate.	1 plate
Hu IL-6 Detection Antibody (100X).Contains 0.1% sodium azide.	0.125 mL
Detection Antibody Diluent. Contains 0.1% sodium azide.	11 mL
Hu IL-6 Standard. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Wash Buffer Concentrate (25X).	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL
Novabright™ CSPD-Emerald II Substrate (clear and greenish)	15 mL
Adhesive Plate Covers	2

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Luminescent microtiter plate reader with software
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before starting

Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **www.lifetechnologies.com/manuals** for details prior to starting the procedure.

Note: Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Dilute wash buffer

- Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
- Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer (1X).
- Store the concentrate and Wash Buffer (1X) in the refrigerator. Use the diluted buffer within 14 days.

Dilute the standards

Note: This assay has been calibrated against the WHO reference preparation 89/548 (NIBSC, Hertfordshire, UK, EN6 3QG). One microgram equals 100,000 units. Use glass or plastic tubes for diluting standards.

- Reconstitute Hu IL-6 Standard to 4000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 4000 pg/mL Hu IL-6. Use the standard within 1 hour of reconstitution.
- Add 450 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 1000, 250, 62.5, 15.63, 3.91, 0.98 and 0 pg/mL Hu IL-6.
- Make 1:4 serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.

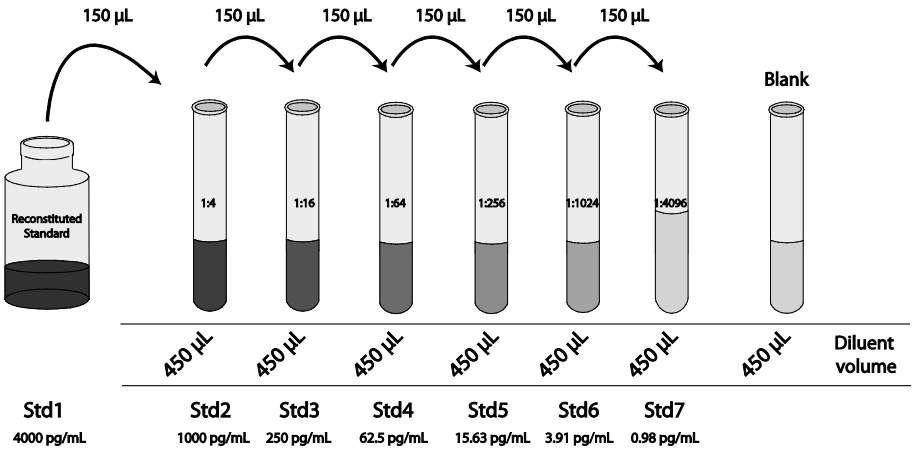
Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.

Prepare Detection Antibody solution

Note: Prepare the Hu IL-6 Detection Antibody solution within 15 minutes of usage.

The Hu IL-6 Detection Antibody (100X) is conjugated to alkaline phosphatase (AP) and is in 50% glycerol, which is viscous. To ensure accurate dilution:

- For each 8-well strip used in the assay, pipet 9 µL Hu IL-6 Detection Antibody (100X) solution, wipe the pipet tip with a clean absorbent paper to remove any excess solution, and dispense the solution to a tube containing 891 µL of Detection Antibody Diluent for a total volume of 900 µL.
- Return the unused Hu IL-6 Detection Antibody (100X) solution to the refrigerator.



Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Samples should be frozen at –80°C if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
- When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Dilute sample

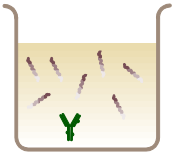
Serum and plasma samples should be diluted 2-fold in Standard Diluent Buffer. Cell culture supernatant samples should be diluted 4-fold in Standard Diluent Buffer.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours and 30 minutes.**

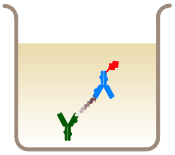
IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.



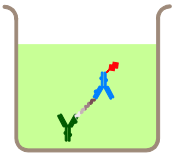
Bind antigen

- Add 100 µL of standards, diluted samples (see page 2) or controls to the appropriate microtiter wells.
- Cover the plate with plate cover and incubate for 2 hours at room temperature.
- Thoroughly aspirate the solution and wash wells 5 times with diluted Wash Buffer.



Add detector antibody

- Add 100 µL Hu IL-6 Detection Antibody (1X) solution into each well.
- Cover the plate with plate cover and incubate for 1 hour at room temperature.
- Thoroughly aspirate the solution from the wells and wash wells 5 times with diluted Wash Buffer.



Add Substrate

- Add 100 µL Novabright™ CSPD-Emerald II Substrate to each well.
- Cover the plate with plate cover and incubate for 30 minutes at room temperature in the dark.

Note: Protect Novabright™ CSPD-Emerald Substrate from prolonged exposure to light.

Read the plate and generate the standard curve

- Read the luminescence (RLU) 30 minutes after the addition of the CSPD-Emerald Substrate with a 1000msec integration time. For best results, keep the plate covered in the dark. Plates should be read as soon as possible after the 30 minutes of substrate incubation.
- Use curve-fitting software to generate the standard curve. A five parameter algorithm with weighting provides the best standard curve fit.
- Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Standard curve (example)

The following data were obtained for the various standards over the range of 0.98–4000 pg/mL Hu IL-6.

Standard Hu IL-6 pg/mL	Luminescence (RLU nm)
4000	287718
1000	111435
250	28682
62.5	6477
15.63	1861
3.91	640
0.98	205
0	95

Specificity

Buffered solutions of a panel of substances at 10 ng/mL were assayed with this Hu IL-6 ELISA kit. The following substances were tested and found to have no cross–reactivity: human : human IL-1a, IL-1ra, IL-1sRI, IL-1sRII, IL-2, IL-3, IL-4, IL-5, IL-6R (soluble), IL-7, IL-8, IL-10, G-CSF, GRO-α, IFN-γ, MIP-1α, MIP-1β, RANTES, TNF-α, TNF-β; mouse IL-1β; rat IL-1β.

Sensitivity

The analytical sensitivity of Hu IL-6 is <0.25 pg/mL. This was determined by adding 2 standard deviations to the mean RLU obtained when the zero standard was assayed 40 times.

The functional sensitivity defines the assay's ability to accurately quantify the lowest known amount of recombinant standard associated with %CV <20%. The functional sensitivity of this assay is ≤2.8 pg/mL.

Performance characteristics

Intra-assay precision

Samples of known Hu IL-6 concentration were assayed in replicates of 24 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	3.0	56.6	950.9
SD	0.27	2.2	59.4
%CV	9	3.9	6.3

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

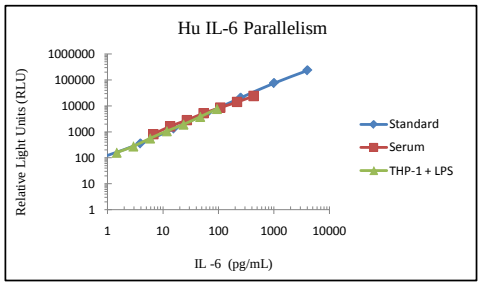
Samples were assayed 72 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	3.4	55.2	918.8
SD	0.3	3.8	75.7
%CV	8.9	6.9	8.3

SD = Standard Deviation; CV = Coefficient of Variation

Parallelism

Natural sample from LPS-treated THP-1 cells and spiked serum were serially diluted in Standard Diluent Buffer. The luminescence (RLU) of each dilution was plotted against the IL-6 standard curve. Parallelism demonstrated by the figure below indicated that the standard accurately reflects IL-6 content in samples. A Logarithmic scale has been used to generate the graph.



Linearity of dilution

Natural sample from LPS-treated THP-1 cells and serum (500µL) were spiked with the recombinant standard (25 µL at 4000 pg/mL), serially diluted in Standard Diluent Buffer over the range of the assay, and measured for Hu IL-6. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99 for spiked serum.

THP-1 cells were grown in tissue culture medium containing 10% fetal bovine serum and treated with 10 µg/mL LPS for 24 hours. Supernatant was collected and diluted in Standard Diluent Buffer. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

	Serum			Supernatant		
Dilution	Measured (pg/mL)	Expected (pg/mL)	% Expected	Measured (pg/mL)	Expected (pg/mL)	% Expected
1/2	305	432	71	90.48	93	97
1/4	170.36	216	79	43.96	47	94
1/8	102.62	108	95	21.95	23	94
1/16	61.52	54	114	11.97	12	103
1/32	33.07	27	123	6.14	6	106

Recovery

Recombinant Hu IL-6 was spiked into serum and plasma to determine percent recovery.

Sample	% Recovery
Serum	105
EDTA Plasma	103
Heparin Plasma	96
Citrate Plasma	102
RPMI + 10% FBS	109

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Lot/Batch code		Protect from light		Use by		Manufacturer
RUO	Research Use Only		Temperature limitation		Consult accompanying documents	EC REP	European Community authorized representative		Directs the user to consult instructions for use (IFU), accompanying the product.