GE Healthcare

Amersham Interleukin-6 [(h)IL-6] Human, Biotrak ELISA System

(96 wells)

Product booklet

Code: RPN2754

Page finder

1. Legal

http://www.gehealthcare.com/lifesciences

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2. Handling

2.1. Safety warnings and precautions

Warning: *For research use only*.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

Store at 2–8°C.

2.3. Expiry

The expiry date is stated on the package and will normally be at least 4 weeks from the date of despatch.

3. Components of the assay system

This pack contains the following assay components, sufficient material for 96 wells.

All reagents are stored refrigerated at 2–8°C. Refer to the expiry date on the kit box.

(h)IL-6 microplate, 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IL-6.

Biotinylated antibody reagent, antibody against human IL-6 conjugated to biotin, with 0.1% (w/v) sodium azide, 8 ml.

(h)IL-6 standard, 2 vials of recombinant human IL-6, lyophilized.

Standard diluent, with 0.1% (w/v) sodium azide, 25 ml.

Streptavidin-HRP concentrate, streptavidin conjugated to horseradish peroxidase, 75 µl.

Streptavidin-HRP dilution buffer, 14 ml.

Wash buffer concentrate, 30-fold concentrated solution, 50 ml.

Pre-mixed TMB substrate reagent, substrate reagent, 12–13 ml.

Stop solution, <1% sulfuric acid, 13–15 ml.

Plate covers, 4 adhesive strips.

4. Additional materials and equipment required

The following materials and equipment are required:

Pipettes or pipetting equipment with disposable tips (50 µl, 100 µl and 1.00 ml)
Disposable polypropylene test tubes - do not use polystyrene, polycarbonate or glass
Measuring cylinder 2 l
Distilled or deionized water
Plate reader capable of reading at 450 nm
A centrifuge for preparing streptavidin-HRP solution

Optional equipment
Assays may be performed with commercially available microplate washers to aid convenience and assay throughput.

5. Description

The Biotrak human interleukin-6 ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (h)IL-6 in cell culture supernatants, plasma, serum and urine.

The assay system is based on a solid phase ELISA, which utilizes an antibody for (h)IL-6 bound to the wells of a microplate together with an antibody to (h)IL-6 conjugated to biotin and streptavidin-HRP detection. Although GE Healthcare (h)IL-6 immunoassay contains recombinant (h)IL-6 and antibodies raised against recombinant (h)IL-6 it has been shown to quantitate accurately both natural (h)IL-6 and recombinant (h)IL-6.

(h)IL-6 can be measured in the approximate range of 10–400 pg/ml (0.5–20 pg/well) in less than 4 hours using the protocol provided with the kit. Each pack contains sufficient material for 96 wells. If one standard curve is constructed, 42 unknowns can be measured in duplicate.

- High sensitivity - <1 pg/ml (0.05 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (h)IL-6

5.1. Summary of the assay

This assay employs the quantitative 'sandwich' enzyme immunoassay technique. An antibody specific for (h)IL-6 has been coated on the microplate provided in the kit. Samples are pipetted into the wells along with biotinylated antibody reagent.

If present, the (h)IL-6 is bound by the immobilized antibody and the biotinylated antibody. After washing away any unbound sample proteins and biotinylated antibody, a streptavidin-HRP conjugate is added to the wells. Any (h)IL-6 which was bound by both the immobilized and the biotinylated antibody during the first incubation will be bound by the streptavidin conjugate. Following a wash to remove unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of (h)IL-6 bound in the initial step.

In addition to the samples to be tested, a series of wells is prepared using known concentrations of the human IL-6 standard. A curve, plotting the optical density versus the concentration of the standard well, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (h)IL-6 in the unknown samples is then determined.

6. Critical parameters

1) Allow samples and all reagents to reach room temperature prior to performing the assays. Do not use water baths to thaw samples or reagents.

2) Mix samples and all reagents thoroughly before use.

3) Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.

4) Avoid handling the tops of the wells both before and after filling.

5) Standards and samples should be assayed in duplicate.

6) Run a separate standard curve for each assay.

7) The total dispensing time for each plate should not exceed 20 minutes.

8) Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.

9) For sample and conjugate incubations a humidified incubator may be used to prevent evaporation loss due to incomplete plate sealing.

10) It is important that the wells are washed thoroughly and uniformly. If using an automatic washer check operation of heads before starting. If washing by hand ensure that all wells are completely filled at each wash.

11) A small amount of precipitate may be present in some vials. It will not affect assay performance and should be ignored.

12) Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metalic azides. Flush with large volumes of water during disposal.

7. Sample preparation

Serum, plasma, urine and cell culture supernatants
Serum, plasma, urine and culture supernatant samples that are to be assayed within 24 hours should be stored at 2–8°C. Specimens to be stored for longer periods of time should be frozen at -70°C to avoid loss of biologically active cytokine. Avoid freezing and thawing samples more than once. Test samples should be assayed in duplicate each time the ELISA is performed, 50 µl of sample per well is required in this way.

Dilution of test samples
If it is suspected that the (h)IL-6 concentration of a sample exceeds the highest point of the standard curve, prepare one or more five fold dilutions of the test sample. Mix thoroughly between dilutions and before assaying.

8. Assay procedure

8.1. Reagent preparation

Wash buffer concentrate
Any precipitate formed during storage will redissolve upon dilution. Dilute 30-fold to prepare 1500 ml of wash buffer. Store at 2–8°C untill the expiry date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

Streptavidin-HRP solution
Prepare the exact amount of streptavidin-HRP solution no more than 15 minutes prior to use.

The streptavidin-HRP concentrate may require spinning down to force the contents to the bottom of the vial. Add 30 µl of streptavidin-HRP concentrate per 12 ml of streptavidin-HRP dilution buffer in a plastic 15 ml tube and mix gently.

If running partial plates use 2.5 µl of streptavidin-HRP concentrate and 1 ml of streptavidin-HRP dilution buffer per strip being run.

Preparation of working standards

a) Two vials of lyophilized standards are provided with this kit. Reconstitute and use one vial per partial plate.

b) Prepare standards shortly before use. Use within one hour of reconstitution.
Do not store reconstituted standards.

c) When **running culture supernatant samples,** reconstitute standard in distilled or deionized water. Reconstitution volume is stated on the standard vial label. The standard will take approximately 1 minute to dissolve. Mix by gently inverting the vial. Use your culture medium to prepare the dilutions of the standard curve, go to step e) below for further instructions. If running a partial plate, refer to step a) above.

d) If **running serum, plasma or urine samples,** reconstitute standard with distilled or deionized water. Reconstitution volume is stated on the standard vial label. The standard will take approximately 1 minute to dissolve. Mix by gently inverting the vial. Use the standard diluent provided to prepare the dilutions of the standard curve. If running a partial plate, refer to step a).

8.2. Preparation of standard curve

1) Label 6 tubes, one for each standard curve point: 400 pg/ml, 160 pg/ml, 64 pg/ml, 25.6 pg/ml, 10.24 pg/ml and 0 pg/ml. Then prepare 1:2.5 serial dilutions for the standard curve as follows:

2) Pipette 240 µl of appropriate diluent (see steps c) and d)) into each tube.

3) Pipette 160 µl of the reconstituted standard into the first tube, 400 pg/ml and mix.

4) Pipette 160 µl of this dilution into the second tube labelled 160 pg/ml and mix.

5) Repeat serial dilutions three more times. These concentrations, 400 pg/ml, 160 pg/ml, 64 pg/ml, 25.6 pg/ml, 10.24 pg/ml and 0 pg/ml are your standard curve.

8.3. Running partial plates

This ELISA provides the flexibility to run two partial plates on separate occasions. Decide the number of strips you wish to run, leaving the strips to be used in the frame. Remove the unnecessary strips and store them in the foil pouch with the desiccant provided at 2–8°C, making sure the foil pouch is sealed tightly.

When adding the TMB substrate reagent, pour out from the bottle **only** the amount needed to run the first half plate. Do not combine left over substrate with that reserved for the second half of the plate. Care must be taken to ensure that the remaining TMB substrate reagent is not contaminated. If the substrate reagent is bright blue prior to use, it has been contaminated.
DO NOT USE.

8.4. Assay protocol

1) Prepare assay reagents and working standards as described in the previous sections.

2) Set up the microplate with sufficient wells to enable the running of all standards and samples as required (see figure 1).

3) Remove excess microplate strips from the frame and store in the resealable foil bag.

4) Add 50 µl of biotinylated antibody to each well that is to be used.

5) Add 50 µl of standard or sample per well. Cover with adhesive strip provided and incubate for 2 hours at room temperature (20–25°C).

6) Aspirate or decant each well and wash, repeating the process twice for a total of three washes. Wash vigorously by filling each well with wash buffer using a washbottle, pipette or manifold dispenser. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towelling.

7) Add 100 µl of pre-diluted streptavidin-HRP solution. Cover with a new adhesive strip and incubate for 30 minutes at room temperature (20–25°C).

8) Repeat the aspiration/wash step as in step 6.

9) Add 100 µl of TMB substrate solution into each well, incubate for 30 minutes at room temperature (20–25°C). If the substrate reagent is bright blue prior to use, do not use. **THE PLATE SHOULD BE DEVELOPED IN THE DARK.** Do not cover the plate with aluminium foil or an adhesive strip.

10) Add 100 µl of stop solution to each well.

11) Determine the optical density of each well within 30 minutes, using a spectrophotometer set to 450 nm.

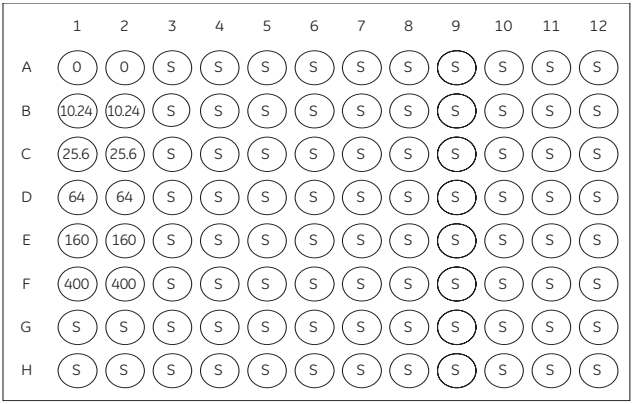

Figure 1. Recommended positioning of standard (0–400 pg/ml) and sample (S) wells.

Summary of assay protocol

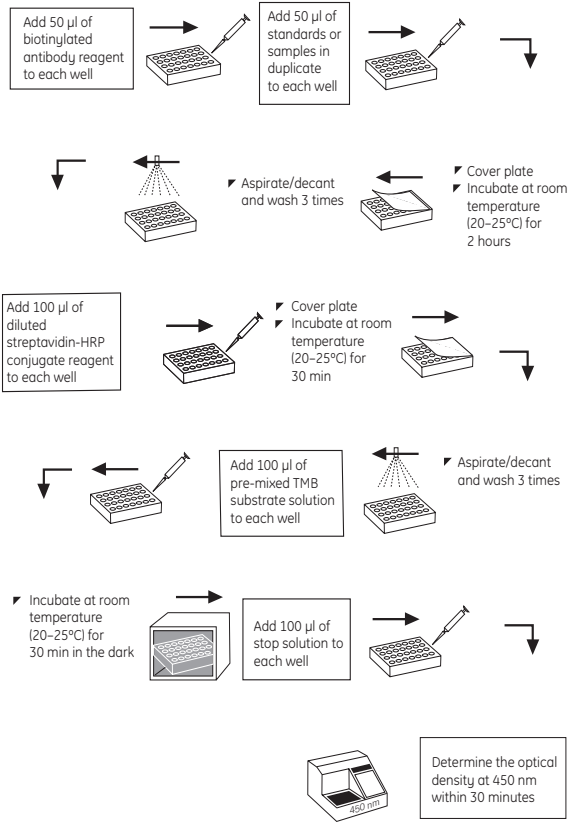

Table 1. Assay protocol (all volumes are in microlitres)

	Zero standard (B_0)	Standards	Samples
Biotinylated antibody reagent	50	50	50
Standard	-	50	–
Standard diluent or cell culture media	50	–	–
Sample	–	–	50
Cover plate, incubate at room temperature for 2 hours.			
Aspirate/decant and wash vigorously all wells three times with wash buffer.			
Streptavidin-HRP conjugate	100	100	100
Cover plate, incubate at room temperature for 30 minutes.			
Aspirate/decant and wash vigorously all wells three times with wash buffer.			
Substrate	100	100	100
Incubate at room temperature for 30 minutes in the dark.			
Stop solution	100	100	100
Determine optical density at 450 nm within 30 minutes.			

9. Data processing

9.1. Calculation of results

Average the duplicate readings for each standard, control and sample and subtract the zero standard optical density.

Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using a log/log plot and regression analysis can be applied to the log transformation.

Figure 2 shows such a plot of the data from table 2. The standard curve is provided for illustration only. A standard curve should be generated for each set of samples to be assayed. This allows for the measurements of 42 unknowns in duplicate.

9.2. Typical assay data

The following data (table 2) were obtained for a standard curve using the protocol provided.

Table 2. Typical assay data

Tube	Optical density	Zero standard subtracted
Zero standard	0.036	-
10.24 pg/ml standard	0.108	0.072
25.6 pg/ml standard	0.209	0.173
64 pg/ml standard	0.407	0.371
160 pg/ml standard	0.904	0.868
400 pg/ml standard	2.164	2.128

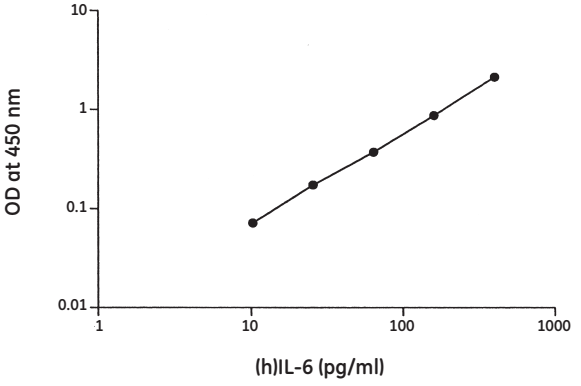

Figure 2. Standard curve

10. Additional Information

10.1. Specificity

This assay recognises both natural and recombinant (h)IL-6. It does not cross react with mouse IL-6, rat IL-6, or human IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-7, IL-8, GM-CSF, TNFα or IFNγ.

10.2. Calibration

The standards in this ELISA are calibrated to the NIBSC reference lot 89/548.
One pg of Biotrak standard = 3 pg of NIBSC standard = 0.3 NIBSC units.

10.3. Reproducibility

Within-assay precision
The within-assay coefficient of variation of the ELISA has been determined to be <10%.
Between-assay precision
The between-assay coefficient of variation of the ELISA has been determined to be <10%.

10.4. Sensitivity

The minimum detectable dose of (h)IL-6 was determined to be <1 pg/ml (0.05 pg/well), by adding two standard deviations to the optical density value of zero and calculating the corresponding concentration from the standard curve.

10.5. Recovery

Serum recovery in the ELISA has been determined by spiking 400 pg/ml recombinant cytokine into neat pooled human serum or plasma and comparing it with spiked standard diluent control. The values overleaf are typical recoveries:

Standard diluent spike	Serum recovery	Plasma recovery
400 pg/ml standard	91%	99%

10.6. Expected values

Sample type and number	Average	Range
Serum (n=14)	43 pg/ml	0–149 pg/ml
Plasma (n=14)	0.7 pg/ml	0–5 pg/ml
Urine (n=5)	0.3 pg/ml	0–0.6 pg/ml

10.7. Background

Unlike many cytokines interleukin-6 is both produced by and has effects on a broad spectrum of cell types. The range of activities include both growth promotion and inhibition, regulation of immunoglobulin and acute phase protein gene expression and induction of differentiation.

Human interleukin-6 ((h)IL-6) is a 212 (precursor), 184 (mature) amino acid protein with a molecular weight of 23–30 kDa (determined by SDS PAGE) which is N-linked glycosylated. Numerous cell types produce IL-6 including: B and T cells and cell lines, monocytes and monocyte lines, fibroblasts, endothelial cells, keratinocytes, bone marrow stromal cells and several tumor cell lines. The IL-6 receptor is a heterodimeric molecule consisting of an 80 kDa IL-6 binding protein and a 130 kDa accessory molecule which is required for the high affinity form (1×10^{-11} M) that can transduce signals. The IL-6 receptors have been found to be on a wide range of cells including B and T cells, monocytes, myelomas, hepatocytes, hepatomas and astrocytomas up to 10 000 per cell.

A soluble circulating form of the 80 kDa IL-6 binding protein is able to form a complex with IL-6 which can then associate with the membrane bound 130 kDa molecule resulting in signal transduction.

Investigations of the role of IL-6 *in vivo* have demonstrated that it is central to several phenomena including: the induction of acute phase response, autoimmune disorders related to polyclonal B cell activation, lymphoid neoplasms, the maturation of megakaryocytes, and the development of plasmacytosis and associated high levels of IgG_1. IL-6 can be induced by a variety of agents including: LPS, IL-1, TNF, IFNβ, calcium ionophores, combinations of mitogenic lectins and phorbol esters and viruses. Dexamethasone has been shown to inhibit IL-6 production by fibroblasts and monocytes.

The deregulation of IL-6 has been implicated in a variety of disease states. Therapeutic strategies for treatment of these diseases therefore focus on disrupting IL-6-mediated effects. IL-6 antagonists may be of value in the management of haematopoietic malignancies, mesangial proliferative glomerulonephritis, B cell abnormalities and several autoimmune diseases.

11. References

1) Van Damme, J. *et al., J. Immunology* **140**, 1534 (1988).
2) Brackenhoff, J.P. *et al., J. Immunology* **139**, 4166 (1987).
3) Hirano, T. *et al., Nature* **324**, 73 (1987).
4) Hirano, T. and Kishimoto, T., *Proc. Nat'l. Acad. Sci.* **82**, 5490 (1985).

12. Related products

Biotrak range of human cytokine ELISA systems

Interleukin-1α [(h)IL-1α]	RPN2750
Interleukin-1β [(h)IL-1β]	RPN2751
Interleukin-2 [(h)IL-2]	RPN2752
Interleukin-10 [(h)IL-10]	RPN2755
Granulocyte-macrophage colony stimulating factor [(h)GM-CSF]	RPN2756
Interferon-gamma [(h)IFN-γ]	RPN2757
Tumour necrosis factor, alpha [(h)TNFα]	RPN2758
Interleukin-8 [(h)IL-8]	RPN2764
Interferon-alpha [(h)IFN-α]	RPN2759

Biotrak range of mouse cytokine ELISA systems

Interleukin-1β [(m)IL-1β]	RPN2720
Interleukin-4 [(m)IL-4]	RPN2712
Tumour necrosis factor-α [(m)TNF-α]	RPN2718

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN20
Cell proliferation ELISA	RPN250
Monoclonal anti-bromodeoxyuridine	RPN202
Cell proliferation labelling reagent	RPN201

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