GE Healthcare

Amersham Interleukin-2 [(h)IL-2] human, Biotrak ELISA system

96 wells

Product Booklet

Code: RPN2752

Page finder

1. Legal

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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. Contents 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-2 microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IL-2.

Biotinylated antibody reagent - antibody against human IL-2 conjugated to biotin, with preservative, 8 ml.

Streptavidin-HRP concentrate - streptavidin conjugated to HRP, with preservative, 50 µl.

Streptavidin-HRP dilution buffer - with preservative, 14 ml.

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

Standard diluent - with preservative, 12 ml.

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

Pre-mixed TMB substrate reagent - with preservative and methanol, 12 ml.

Stop solution - 0.18 M sulphuric acid, 15 ml.

Plate covers - 4 adhesive strips.

4. Description

The Biotrak™ human interleukin-2 ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (h)IL-2 in cell culture supernatants, serum and plasma.

The assay system is based on a solid phase ELISA, which utilizes an antibody for (h)IL-2 bound to the wells of a microplate together with an antibody to (h)IL-2 conjugated to biotin and streptavidin-HRP detection. Although the Biotrak (h)IL-2 immunoassay contains recombinant (h)IL-2 and antibodies raised against recombinant (h)IL-2 it has been shown to quantitate accurately both natural (h)IL-2 and recombinant (h)IL-2.

(h)IL-2 can be measured in the approximate range 38–1500 pg/ml (1.9–75 pg/well) in less than 5 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 - <5 pg/ml (0.25 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (h)IL-2

5. Introduction

Originally named T cell growth factor, IL-2 was first characterized as a factor present in mitogen-stimulated leukocyte supernatants which was capable of supporting long term cultures of T lymphocytes. IL-2 has since been shown to play a pivotal role in the clonal expansion of antigen-specific T cells during the immune response. In addition to its role as an autocrine growth factor for T-cells, IL-2 also upregulates T cell and NK cell cytolytic activity, promotes B cell proliferation and differentiation and induces the synthesis of IL-1, IL-6, TNF and IFN gamma by peripheral blood mononuclear cells.

IL-2 is a 133 amino acid protein of 15-17.5 kDa containing variable 0-linked sialylation (which is not required for activity). IL-2 production is restricted to T cells, NK cells, certain B cell tumours and, possibly, activated B cells. The synthesis of IL-2 is induced by PHA, PMA, calcium ionophores, anti-CD2, -CD3, and -CD28 mAbs, IL-1, IL-7, antigenic stimulation of TCRs and diacyl glycerol analogues. IL-2 synthesis is inhibited by cyclosporin A, FK-506, cAMP, hydrocortisone and prostaglandins.

IL-2 has been shown to induce the rejection of cardiac and skin allografts in rat models and to enhance the immune response to bacterial and viral infections. Elevated levels of circulating IL-2 have been detected in a variety of autoimmune disorders including systemic lupus erythematosis, scleroderma, multiple sclerosis and rheumatoid arthritis. IL-2 also mediates tumour regression in several mouse models.

There are three types of IL-2 receptor (IL-2R) which arise from the combination of three subunits. These subunits consist of two distinct IL-2 binding proteins (IL-2Rα, p55; IL-2Rβ, p75) and a third accessory protein (IL-2Rγ, p64). The three types of IL-2R differ in affinity and signalling capability. The low affinity, αIL-2R (10 nM) binds without signalling. In contrast, both the intermediate affinity, $\beta+\gamma$ IL-2R (1 nM)

and the high affinity $\alpha+\beta+\gamma$ IL-2R (10 pM) mediate internalization and signal transduction. The gamma chain is believed to be essential for IL-2 signalling.

The ability of IL-2 to upregulate tumouricidal activity has led to its evaluation as an anti-neoplastic agent for a variety of malignancies. IL-2 therapy may also prove to be useful in immune-deficiency disorders in which endogenous IL-2 levels are greatly reduced.

6. Summary of the assay

This assay employs the quantitative 'sandwich' enzyme immunoassay technique. An antibody specific for (h)IL-2 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-2 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-2 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 colour develops in proportion to the amount of (h)IL-2 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-2 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-2 in the unknown samples is then determined.

7. Assay methodology

Users are recommended to read this entire section before starting work.

7.1. 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 preparation of streptavidin-HRP

Optional equipment

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

7.2. Sample preparation

Serum, plasma and cell culture supernatants

Serum, plasma and cell culture supernatant 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.

Dilution of test samples

If you suspect that the (h)IL-2 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.

* GE Healthcare supplies a range of pipettes and disposable tips (see related products).

7.3. Critical parameters

1. First select the number of strips to be run and 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 help 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.

7.4. Assay procedure

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 until 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 or plasma**, 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) above.

e. Label 6 tubes, one for each standard curve point: 1500 pg/ml, 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml and 0 pg/ml. Then prepare 1:2.5 serial dilutions for the standard curve as follows:

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

g. Pipette 160 µl of the reconstituted standard into the first tube, 1500 pg/ml and mix.

h. Pipette 160 µl of this dilution into the second tube labelled 600 pg/ml and mix.

i. Repeat serial dilutions three more times. These concentrations, 1500 pg/ml, 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml and 0 pg/ml are your standard curve.

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.**

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 3 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. Aspirate or decant each well, 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 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.

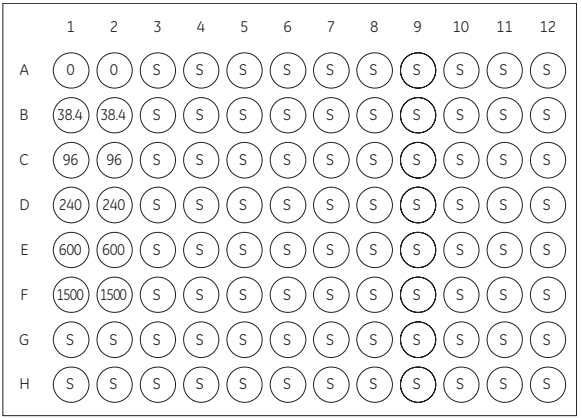
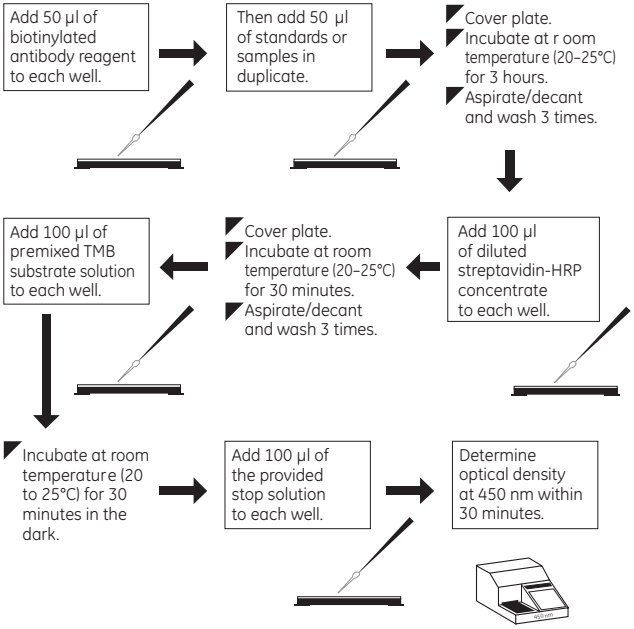


Figure 1. Recommended positioning of standard (0–1500 pg/ml) and sample wells (S).

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

	Zero Standard (B_0)	Standards	Samples
Biotinylated antibody	50	50	50
Standard	-	50	-
Standard diluent or cell culture media	50	-	-
Sample	-	-	50
Cover plate, incubate at room temperature for 3 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.			

Summary of assay protocol



7.5. 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 may 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.

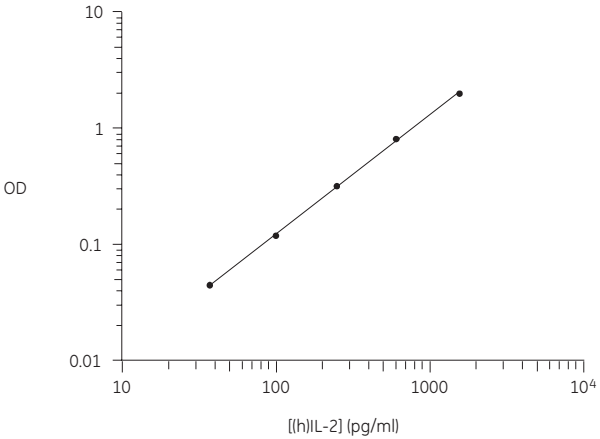


Figure 2. Standard curve

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	0.037	
38.4 pg/ml standard	0.081	0.044
96 pg/ml standard	0.149	0.112
240 pg/ml standard	0.342	0.305
600 pg/ml standard	0.850	0.813
1500 pg/ml standard	1.949	1.912

8. Additional information

8.1. Specificity

This assay recognizes both natural and recombinant (h)IL-2. It does not cross react with (m)IL-2, (h)IL-1α, (h)IL-1β, (h)IL-3, (h)IL-4, (h)IL-6, (h)IL-7, (h)IL-8, (h)IFNγ or (h)TNFα.

8.2. Calibration

The standard in this ELISA is calibrated to the NIBSC reference lot 86/504.
One (1) pg of Biotrak standard = 2 NIBSC pg.

8.3. 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%.

8.4. Sensitivity

The minimum detectable dose of (h)IL-2 was determined to be <6 pg/ml (0.3 pg/well), by adding two standard deviations to the optical density value of zero and calculating the corresponding concentration from the standard curve.

8.5. Recovery

Recovery in the ELISA has been determined by spiking recombinant cytokine into cell culture media, normal human serum, plasma and controls at three different levels and running these samples in the ELISA. The values below are typical recoveries:

Control	Mean plasma recovery
100 pg/ml	100%
275 pg/ml	99%
400 pg/ml	90%

Control	Mean plasma recovery
100 pg/ml	101%
275 pg/ml	106%
400 pg/ml	86%

8.5. Expected values

	Average	Range
Normal serum	1.0 pg/ml	0–4.0 pg/ml
Normal plasma	3.6 pg/ml	0–6.5 pg/ml

9. References

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2. JORDAN, S.C. *et al., J. Heart Lung Transplant*, **12(2)**, p.333, 1993.

3. DECKERT-SCHLUTER, M. *et al., J. Neurol. Sci.* **113(1)**, p.50, 1992.

4. NEEDLEMAN, B.W. *et al., Arthritis Rheum. (USA)* **35(1)**, p.67, 1992.

5. NOGUCHI, K. *et al., Transplant Proc.* **24(3)**, p.115, 1992.

6. WU, C.J. *et al., Transplantation* **54(2)**, p.326, 1992.

7. BERNELL, P. *et al., Hematol Oncol. (England)* **9(3)**, p.129, 1991.

8. PETER, J.B. *et al., Neurology* **41(1)**, p.121, 1991.

9. SIMPSON, M.A. *et al., Clin. Lab. Med.* **11(3)**, p.733, 1991.

10. Related products

Biotrak range of human cytokine ELISA systems	
Interleukin-1α [(h)IL-1α]	RPN 2750
Interleukin-1β [(h)IL-1β]	RPN 2751
Interleukin-4 [(h)IL-4]	RPN 2753
Interleukin-6 [(h)IL-6]	RPN 2754
Interleukin-10 [(h)IL-10]	RPN 2755
Granulocyte-macrophage colony stimulating factor [(h)GM-CSF]	RPN 2756
Interferon-gamma [(h)IFNγ]	RPN 2757
Tumour necrosis factor, alpha [(h)TNFα]	RPN 2758
Interferon-alpha [(h)IFNα]	RPN 2759
Interleukin-8 [(h)IL-8]	RPN 2760
Biotrak range of mouse cytokine ELISA systems	
Interleukin-1α [(m)IL-1α]	RPN 2719
Interleukin-1β [(m)IL-1β]	RPN 2720
Interleukin-2 [(m)IL-2]	RPN 2710
Interleukin-3 [(m)IL-3]	RPN 2711
Interleukin-4 [(m)IL-4]	RPN 2712
Interleukin-5 [(m)IL-5]	RPN 2713
Interleukin-6 [(m)IL-6]	RPN 2714
Interleukin-10 [(m)IL-10]	RPN 2715
Granulocyte-macrophage colony stimulating factor [(m)GM-CSF]	RPN 2716
Interferon-gamma [(m)IFNγ]	RPN 2717
Soluble intercellular adhesion molecule-1 [(m)sICAM-1]	RPN 2721
Tumour necrosis factor-α [(m)TNF-α]	RPN 2718

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

Cell proliferation assay system	RPN 210
Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN 20
Monoclonal anti-bromodeoxyuridine	RPN 202
Cell proliferation labelling reagent	RPN 201

Pipettes and pipette tips

Single channel, variable volume pipettes	
Volume range	
0.5–10 µl	RPN 2340
5–50 µl	RPN 2341
50–200 µl	RPN 2342
200–1000 µl	RPN 2343
1–5 ml	RPN 2344
Multi-channel, variable volume pipettes	
8 channel, 5–50 µl	RPN 2372
8 channel, 50–250 µl	RPN 2373

GE Healthcare's range also includes fixed volume, 4 and 12 multi-channel variable volume pipettes, a range of pipette tips and related accessories.

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