GE Healthcare

Amersham Interleukin-2 [(m)IL-2] Mouse, Biotrak ELISA System

96 wells

Product Booklet

Code: RPN2710

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 and provides sufficient material for 96 wells.

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

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

(m)IL-2 conjugate - antibody against mouse IL-2 conjugated to horseradish peroxidase, with preservative, 100 µl.

Conjugate dilution buffer - diluent, with preservative, 14 ml.

(m)IL-2 standard - recombinant mouse IL-2, lyophilized, 2 vials.

Plate reagent - with 0.1% (w/v) sodium azide, 12 ml.

Standard diluent - with 0.1% (w/v) sodium azide, 12 ml.

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

Pre-mixed TMB substrate solution - substrate solution, with preservative and methanol, 13 ml.

Stop solution - 0.18 M sulphuric acid.

Plate covers - adhesive strips, 4.

4. Critical parameters

- Allow samples and all reagents to reach room temperature prior to performing the assays. Do not use water baths to thaw samples or reagents.
- Mix samples and all reagents thoroughly before use.
- Avoid excessive foaming of reagents. Also avoid exposure of reagents to excessive heat or light during storage and incubation.
- Avoid handling the tops of the wells both before and after filling.
- Standards and samples should be assayed in duplicate.
- Run a separate standard curve for each assay.
- The total dispensing time for each plate should not exceed 20 minutes.
- Use only coated wells from the same reagent batch for each assay. Also do not mix reagents from different kit lots.
- For sample and conjugate incubation a humidified incubator may be used to help prevent evaporation loss due to incomplete plate sealing.
- Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

5. Description

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

The assay system is based on a solid phase ELISA which utilizes an antibody for (m)IL-2 bound to the wells of a microplate (12 x 8 well strip format) together with an antibody to (m)IL-2 conjugated to horseradish peroxidase. Although the Biotrak (m)IL-2 immunoassay contains recombinant (m)IL-2 and antibodies raised against recombinant (m)IL-2 it has been shown to quantitate accurately both natural (m)IL-2 and recombinant (m)IL-2.

(m)IL-2 can be measured in the range 34–850 pg/ml (1.7–42.5 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, 44 unknowns can be measured in duplicate.

- High sensitivity - 3 pg/ml (0.15 pg/well)
- Same day protocol
- Pre-coated microplate
- Specific for (m)IL-2

6. Summary of the assay

This assay employs the quantitative 'sandwich' enzyme immunoassay technique. An antibody specific for (m)IL-2 has been coated on to the microplate provided in the kit. Samples are pipetted into the wells, and the (m)IL-2, if present, is bound by the immobilized antibody. After washing away any unbound sample proteins, an enzyme-linked antibody specific for (m)IL-2 is added to the wells and allowed to bind to any (m)IL-2 which was bound during the first incubation. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of (m)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 (m)IL-2 standard. A curve, plotting the optical density versus the concentration of (m)IL-2 in these standard wells, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (m)IL-2 in the unknown samples is then determined.

7. 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
- Reagent reservoirs for use with multi-channel pipettes

Optional equipment

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

8. Sample preparation

Cell culture supernatants
Centrifuge to remove any particulate material and store at -15°C to -30°C.
Avoid freeze-thaw cycles.

Serum
Serum samples should be allowed to clot at room temperature. Immediately after clotting, spin down. Specimens should be clear and non-haemolysed whenever possible. If samples contain particulate matter, clarify by centrifugation before testing.

Serum 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.

Dilution of test samples
If it is suspected that the (m)IL-3 concentration of a sample exceeds the highest point of the standard curve, one or more five-fold dilutions of the test sample should be prepared. Mix thoroughly between dilutions and before assaying.

9. Assay procedure

9.1. Reagent preparation

Wash buffer concentrate

Any precipitate formed during storage will redissolve upon dilution. Dilute 30-fold with distilled or deionized water 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.

Conjugate dilution

Prepare conjugate no more than 15 minutes prior to use. Do not prepare more conjugate than required. Do not store prepared conjugate. Prepare the conjugate by adding 66 µl of conjugate concentrate to 11 ml of conjugate dilution buffer in a 15 ml plastic tube.

If running partial plates use 6 µl of conjugate concentrate and 1 ml of conjugate dilution buffer for each strip being run.

m)IL-2 standard

It is important that the diluent selected for reconstitution and dilution of the standard reflects the environment of the samples being measured. Standard diluent will be suitable for the serial dilution of standards for serum determinations. For cell culture supernatant samples, the culture media will be suitable for preparation of the standard curve.

Reconstitute the (m)IL-2 standard with distilled or deionized water for serum samples, and culture media for cell culture supernatants. Reconstitution volume is stated on the standard vial label. This reconstitution produces a stock solution of 850 pg/ml. Mix by gently inverting the vial. Use this stock solution to produce a dilution series, as described below, within the range of this assay (34–850 pg/ml). Use standards within 15 minutes of dilution.

9.2. Preparation of standard curve

The reconstituted 850 pg/ml (m)IL-2 is the first point of the standard curve. For the other points, prepare five-fold serial dilutions as follows: label two tubes, one tube for each of the additional dilutions: 170 pg/ml and 34 pg/ml. Pipette 400 µl of appropriate diluent into each tube. Pipette 100 µl of reconstituted (m)IL-2 standard into the first tube labelled 170 pg/ml and mix. Pipette 100 µl of this dilution into the second tube labelled 34 pg/ml and mix. These concentrations, 850 pg/ml, 170 pg/ml and 34 pg/ml provide the standard curve points.

9.3. Running partial plates

This ELISA provides the flexibility to run two partial plates on separate occasions. Decide the number of strips required 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 solution, pour out from the bottle **only** the amount needed to run a partial plate. Do not combine left over substrate with that reserved for the remainder of the plate. Care must be taken to ensure that the remaining TMB substrate solution is not contaminated. If the substrate solution is bright blue prior to use, it has been contaminated. **DO NOT USE.**

9.4. 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 with the desiccant provided.

4. Add 50 µl of plate reagent to each well that is to be used.
5. Add 50 µl of standard or sample per well in duplicate. Cover with adhesive strip provided and incubate for 2 hours at 37°C ± 2°C.
6. Aspirate or decant each well and wash, repeating the process four times for a total of five washes. Wash vigorously by filling each well with wash buffer (400 µl) 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. Squeeze the sides of the plate when decanting to ensure that all strips remain securely in the frame.
7. Add 100 µl of (m)IL-2 conjugate. Cover with a new adhesive strip and incubate for 1 hour at 37°C ± 2°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. If the substrate solution 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.
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.

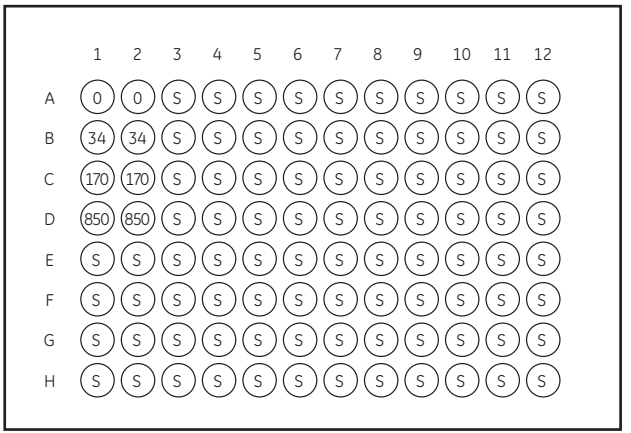

Figure 1. Recommended positioning of standard (0–850 pg/ml) and sample wells (S)

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

	Zero standard (B_0)	Standards	Samples
Plate reagent	50	50	50
Standard	–	50	–
Standard diluent or cell culture media	50	–	–
Sample	–	–	50
Cover plate, incubate at 37°C ± 2°C for 2 hours.			
Aspirate/decant and vigorously wash all wells five times with 400 µl wash buffer.			
Conjugate	100	100	100
Cover plate, incubate at 37°C ± 2°C for 1 hour.			
Aspirate/decant and vigorously wash all wells five times with 400 µl 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.			

10. Data processing

10.1. Calculation of results

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

Plot these averaged absorbance values for each of the standard values versus the corresponding concentration of the standards. 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, with 7 points plotted, is provided for illustration purposes only. A standard curve should be generated for each set of samples to be assayed and the protocol describes the generation of a 4 point curve (zero included) by dilution of a stock standard. This allows for the measurements of 44 unknowns in duplicate.

If a test sample has been diluted prior to assay, account for the dilution factor in calculation of results

10.2. Typical assay data

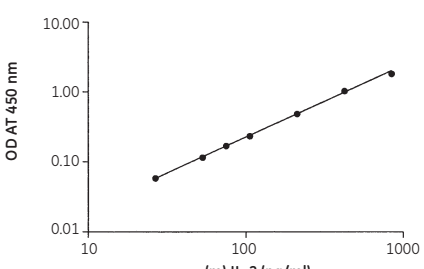

Figure 2. Standard curve

The following data (table 2) were obtained for a standard curve using the protocol provided, with an extended dilution series to demonstrate the linearity of the assay curve.

Table 2. Typical assay data

Tube	Optical density	Zero standard subtracted
Zero standard	0.032	–
26.6 pg/ml standard	0.089	0.058
53.1 pg/ml standard	0.146	0.115
75 pg/ml standard	0.199	0.168
106.2 pg/ml standard	0.264	0.232
212.5 pg/ml standard	0.510	0.479
425 pg/ml standard	1.057	1.026
850 pg/ml standard	1.839	1.807

11. Additional information

11.1. Specificity

This assay recognizes both natural and recombinant (m)IL-2. It does not cross react with (h)IL-1α, (h)IL-2, (h)IL-3, (h)IL-6, (h)IL-8, (h)IFNγ, (h)TNFα, (m)IL-3, (m)IL-4, (m)IL-5, (m)IL-6, (m)IL-10, (m)GM-CSF, (m)IFNγ, or (m)TNFα.

11.2. 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%.

11.3. Sensitivity

The minimum detectable dose of biologically active (m)IL-2 was determined to be 3 pg/ml (0.15 pg/well), by adding two standard deviations to the optical density value of zero and calculating the corresponding concentration from the standard curve.

11.4. Recovery

Serum recovery in the ELISA has been determined by spiking recombinant cytokine into neat pooled mouse serum and comparing it with spiked standard diluent control. The values below are typical recoveries:

Sample type	Value	Recovery
Control	92.8 pg/ml	n/a
Serum	120 pg/ml	129%
Cell culture supernatant	121 pg/ml	130%

11.5. Background and references

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 IFNγ by peripheral blood mononuclear cells.

IL-2 is a 133 amino acid protein of 15–17.5 kDa containing variable O-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 monoclonal antibodies, 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 signaling capability. The low affinity, IL-2R (α,10nM) binds without signalling. In contrast, both the intermediate affinity, IL-2R (β + γ,1nM)

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

The ability of IL-2 to up-regulate tumoricidal 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.

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11.6.Related products

Human cytokine ELISA systems from the Biotrak assay range

Unlabelled and radiolabelled growth factors and cytokines

Cell proliferation

Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN 20
Cell proliferation ELISA	RPN 250
Monoclonal anti-bromodeoxyuridine	RPN 202
Cell proliferation labelling reagent	RPN 201

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