GE Healthcare

Amersham Interleukin-1 Beta [(m)IL-1β] Mouse, Biotrak ELISA System

96 wells

Product Booklet

Code: RPN2720

Page finder

1. Legal

Contact your local GE Healthcare representative for the most current information.

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.

(m)IL-1β microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against mouse IL-1β.

Biotinylated antibody reagent - antibody against mouse IL-1β conjugated to biotin, 8 ml.

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

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

(m)IL-1β standard - 2 vials of recombinant mouse IL-1β, lyophilized.

Standard diluent - with preservative, 12 ml.

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

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

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

Plate covers - 4 adhesive strips.

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.

5. Description

The Biotrak™ mouse interleukin-1β ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (m)IL-1β in cell culture supernatants, serum and plasma.

The assay system is based on a solid phase ELISA, which utilizes a monoclonal antibody for (m)IL-1β bound to the wells of a microplate together with a biotinylated antibody to (m)IL-1β and streptavidin conjugated to horseradish peroxidase. Although the Biotrak (m) IL-1β immunoassay contains recombinant (m)IL-1β and antibodies raised against recombinant (m)IL-1β it has been shown to quantitate accurately both natural (m)IL-1β and recombinant (m)IL-1β.

(m)IL-1β can be measured in the approximate range 15.6–1000 pg/ml (0.78–50 pg/well) in less than 3.5 hours using the protocol provided with the kit. Each pack contains sufficient material for 96 wells. If one standard curve is constructed, 43 unknowns can be measured in duplicate.

- High sensitivity – 3 pg/ml (0.15 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (m)IL-1β

5.1. Summary of the assay

This assay employs a quantitative *in vitro* enzyme linked immunoabsorbent technique. A monoclonal antibody specific for (m) IL-1β has been coated on the microplate provided in the kit. Samples are pipetted into the wells along with biotinylated antibody reagent. If present, the (m)IL-1β is bound by both the immobilized and the biotinylated antibody. After washing away any unbound sample proteins and biotinylated antibody, a streptavidin-HRP conjugate is added to the wells. Any (m)IL-1β 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 any unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of (m)IL-1β 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-1β standard. A curve, plotting the optical density versus the concentration of (m)IL-1β in these standard wells, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (m) IL-1β in the unknown samples is then determined.

6. 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 cylinders 2 l and 20 ml
- Distilled or deionized water
- Plate reader capable of reading at 450 nm

Optional equipment

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

7. 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-hemolyzed whenever possible. If samples contain particulate matter, clarify by centrifugation before testing. Serum, plasma and cell 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.

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 up to the expiry date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

Streptavidin-HRP solution

No more than 15 minutes prior to use, prepare the exact quantity of streptavidin-HRP solution required. Do not store prepared streptavidin-HRP solution. The streptavidin-HRP concentrate may require spinning down to force the entire contents to the bottom of the vial. Add 30 µl of streptavidin-HRP concentrate to 12 ml streptavidin-HRP dilution buffer in a 15 ml plastic tube and mix gently. The streptavidin-HRP solution is now ready for use.

If running partial plates, use only the amount of streptavidin-HRP solution required for the number of strips being run. Use 2.5 µl of streptavidin-HRP concentrate and 1 ml of streptavidin-HRP dilution buffer per strip being run.

(m)IL-1β standards

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 dilution in serum and plasma measurements. If the samples are cell culture supernatants, culture media will be suitable for preparation of the standard curve.

Reconstitute the (m)IL-1β standard with distilled or deionized water (reconstitution volume is stated on the standard vial label).

This reconstitution produces a stock solution of 4000 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 (15–1000 pg/ml). Use standards within 60 minutes of dilution.

Testing of RPMI with different concentrations of fetal bovine serum has shown that this assay is not adversely affected by culture medium. Therefore when running both culture supernatants and serum or plasma samples on the same plate, reconstitute the standard with water and carry out the dilutions in standard diluent. If you are using an unusual type of culture medium, you may wish to validate the medium by running two standard curves in parallel; one diluted in standard diluent and one diluted in culture medium. If the two standard curves are within 10% of the mean for both standard curves then the assay can be run using either curve.

8.2. Preparation of the standard curve

Label five tubes, one tube for each standard dilution: 1000 pg/ml, 250 pg/ml, 62.5 pg/ml, 15.6 pg/ml and 0 pg/ml. Pipette 300 µl of appropriate diluent into each tube. Pipette 100 µl of reconstituted (m)IL-1α standard into the first tube labelled 1000 pg/ml and mix. Pipette 100 µl of this dilution into the second tube labelled 250 pg/ml and mix. Repeat this serial dilution **twice** more to give a standard concentration range of 1000–15.6 pg/ml. These concentrations, 1000 pg/ml, 250 pg/ml, 62.5 pg/ml and 15.6 pg/ml, together with the zero standard (0 pg/ml), which contains only appropriate diluent, are used to provide the standard curve points.

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 the biotinylated antibody reagent to all wells that will be used.
5. Add 50 µl of reconstituted standard or sample per well, adding 50 µl of standard diluent or cell culture media to the zero standard wells. 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 (~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.

7. Add 100 µl of streptavidin-HRP conjugate. 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.

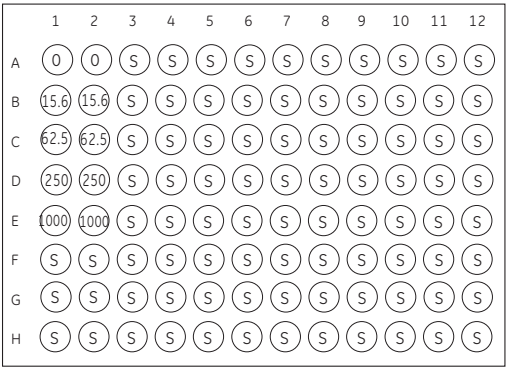

Figure 1. Recommended positioning of standard (0–1000 pg/ml) and sample wells (S).

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 (20°C–25°C) for 2 hours.			
Aspirate/decant and wash vigorously all wells three times with ~400 µl wash buffer.			
Streptavidim- HRT concentrate	100	100	100
Cover plate, incubate at room temperature (20–25°C) for 30 minutes.			
Aspirate/decant and wash vigorously all wells three times with ~400 µl wash buffer.			
Substrate	100	100	100
Incubate at room temperature (20–25 °C) 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 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 measurement of 43 unknowns in duplicate.

9.2. Typical assay data

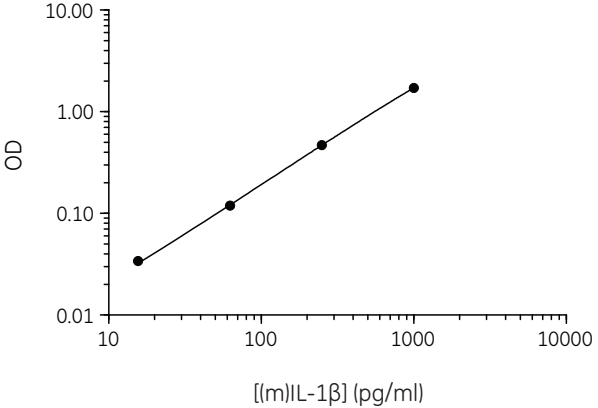

Figure 2. Standard curve

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	0.024	–
15.6 pg/ml standard	0.058	0.034
62.5 pg/ml standard	0.143	0.119
250 pg/ml standard	0.493	0.459
1000 pg/ml standard	1.739	1.715

10. Additional information

10.1. Specificity

This assay recognizes both natural and recombinant (m)IL-1β. It does not cross- react with (h)IL-1β, (m)IL-1α, (m)IL-3, (m)IL-4, (m)IL-5, (m) IL-7, (m)IL-10, (m)GM-CSF, (m)TNFα, or (m)IFNγ.

10.2. Calibration

The standard in this ELISA is calibrated to the NIBSC reference lot 93/668
One (1) pg of Biotrak standard = 0.8 NIBSC pg

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 (m)IL-1β was determined to be <2 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.

10.5. Parallelism

The linearity of dilution was determined by serially diluting seven different positive samples. The dilutions were run in the assay and the found doses were plotted against the expected doses.

10.6. Recovery

Recovery in this assay has been determined by spiking recombinant cytokine into normal mouse serum and comparing it with a control. This was also carried out in various types of plasma. An average recovery in serum was 89% ± 7.6%, and average recovery in plasma was 86% ± 8%.

10.7. Expected values

The average levels of (m)IL-1β found in 31 normal serum samples was 12pg/ml with a range of 0–82 pg/ml. The average level of (m) IL-1β found in 12 normal plasma samples was 1.6 pg/ml with a range of 0–16 pg/ml.

10.8. Background and references

IL-1β is one of a group of three related polypeptide hormones that also includes IL-1α and IL-1ra (receptor antagonist). The IL-1 molecules exert effects on a variety of cell types involved in the host response to injury and infection.

IL-1β is produced from a 269 amino acid precursor which is not biologically active. The mature form of IL-1β is a 153 amino acid protein of 17.5 kDa (determined by SDS-PAGE) which is produced from the precursor by (m)IL-1β protease. Although many cell types synthesize the IL-1β precursor, expression of the IL-1β protease appears to be limited to monocytes and myeloid cell lines.

IL-1β can be induced by a variety of agents including: LPS, *S. aureus*, IL-1, leukotrienes, phorbol esters, TNF, C5a, GM-CSF, indomethacin and zymosan. Agents that inhibit IL-1β production include: dexamethasone, predisolone, cAMP, PGE_2, IL-4, IL-10, TGFβ , and retinoic acid.

Two distinct IL-1 receptors (IL-1R) have been identified, termed

type I (80 kDa) and type II (68 kDa). Both receptors bind IL-1α and IL-1β with high affinity (~10^{-10} M). IL-1RI is expressed by T cells, endothelial cells and fibroblasts while IL-1RII is expressed by B cells and other monomyelocytic cell types. Receptor density ranges from 0–30 000 per cell with fibroblasts and keratinocytes displaying the highest levels. Both IL-1RI and IL-1RII are members of the Ig superfamily.

Although IL-1 has been demonstrated to play an important role in several *in vivo* phenomena, the detection of IL-1 *in vivo* using bioassays has been difficult. IL-1 is an endogenous pyrogen and induces fever in animal models. IL-1 is also involved in muscle proteolysis, bone resorption, wound healing, hematopoiesis and inflammatory diseases, including diabetes, periodontitis and rheumatoid arthritis.

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10.9. Related products

Biotrak range of mouse cytokine ELISA systems

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

Biotrak range of human cytokine ELISA systems
Please contact your local GE Healthcare office for full details

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

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

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