GE Healthcare

# Amersham Interleukin-1α [(r)IL-1α], Rat, Biotrak ELISA System

96 wells

## Product Booklet

Code: RPN2735

# Page finder

# 1. Legal



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GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK

# 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°C–8°C.

## 2.3. Expiry

The expiry date is stated on the package and will 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.

**(r)IL-1α microplate** - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against rat IL-1α.

**(r)IL-1α standard** - 2 vials of E.*coli*-derived rat recombinant IL-1α, lyophilized.

**Standard diluent** - with preservative, 12 ml.

**Biotinylated antibody reagent** - antibody against (r)IL-1α conjugated to biotin, with preservative, 12 ml.

**Streptavidin-HRP concentrate** - streptavidin conjugated to HRP, with preservative, 75 µl.

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

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

**Pre-mixed TMB substrate reagent** - with preservative, 13 ml.

**Stop solution** - 0.18 M sulphuric acid, 14 ml.

**Plate covers** - 6 adhesive strips.

# 4. Description

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

The assay is based on a solid phase ELISA, which utilizes an antibody for (r)IL-1α bound to the wells of a microplate together with a biotinylated antibody to (r)IL-1α and streptavidin conjugated to horseradish peroxidase (figure 1). Although GE Healthcare's (r)IL-1α immunoassay contains recombinant (r)IL-1α and antibodies raised against recombinant (r)IL-1α it has been shown to quantitate accurately both natural (r)IL-1α and recombinant (r)IL-1α.

(r)IL-1α can be measured in the range 51.2–2000 pg/ml (5.12–200 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 <15 pg/ml (1.5 pg/well)
- Total incubation time of 4 hours
- Pre-coated plate
- Specific for (r)IL-1α
- Range 51.2–2000 pg/ml
- Small sample size, 100 µl per well

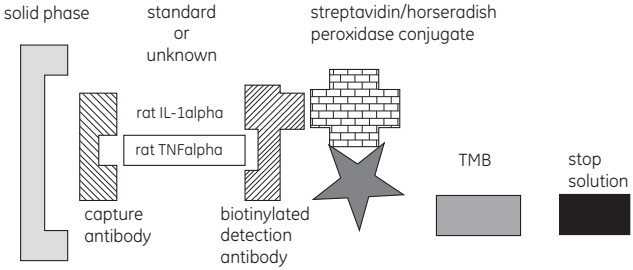


**Figure 1.** Rat interleukin-1 alpha ELISA assay design

# 5. 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.
- It is important that the wells are washed thoroughly and uniformly. If using an automatic washer check operation of head before starting. If washing by hand ensure that all wells are completely filled at each wash.
- A small amount of precipitate may be present in some vials. It will not affect assay performance and should be ignored.
- Use a new adhesive plate cover for each incubation step in the ELISA.
- New pipette tips should be used for each standard and sample. GE Healthcare recommends the use of multiple tips or multi-shot dispensing pipettes for the addition of other reagents to the wells.

# 6. Additional materials and equipment required

The following materials and equipment are required:

- Pipettes or pipetting equipment with disposable tips (30 µl, 100 µl, 160 µl, 240 µl and 1.0 ml)
- Disposable polypropylene test tubes - do not use polystyrene, polycarbonate or glass
- 15 ml plastic test tube
- Equipment for dispensing 12 ml
- Measuring cylinder and 2 l container
- Distilled or deionized water
- Plate reader capable of reading at 450 nm
- A bench top centrifuge for preparing the streptavidin-HRP solution
- Wash bottle or automatic plate washer

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

# 7. Specimen collection and sample preparation

## 7.1. Serum, plasma and cell culture supernatants

Serum, plasma 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, 100 µl of sample per well is required in this way.

**Cell culture supernatant**
Centrifuge to remove any particulate matter.

**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.

**Dilution of test samples**
If it is suspected that the (r)IL-1α concentration of a sample exceeds the highest point of the standard curve, prepare one or more five-fold dilutions of the test sample. If running culture supernatants prepare the dilutions using your medium. If running either serum or plasma prepare the dilutions using the standard diluent provided. Mix thoroughly between dilutions and before assaying.

# 8. Elisa 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 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. Do not prepare more conjugate than required. Do not store diluted conjugate.

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 buffer per strip being run.

## 8.2. Preparation of working standards

1. Two vials of lyophilized standard are provided with this kit. Reconstitute and use one vial per partial plate.
2. Prepare standards shortly before use. Use within one hour of reconstitution.
   **Do not store reconstituted standards.**
3. If **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 the culture medium to prepare the dilutions of the standard curve,** go to

step 5 below for further instructions. If running a partial plate, refer to step 1.

4. **If running serum, or plasma 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 1.
5. Label 6 tubes, one for each standard curve point: 2000 pg/ml, 800pg/ml, 320 pg/ml, 128 pg/ml, 51.2 pg/ml and 0 pg/ml. Then prepare 2.5 fold serial dilutions for the standard curve as follows:
6. Pipette 480 µl of appropriate diluent (see steps 3 and 4 above) into each tube.
7. Pipette 320 µl of the reconstituted standard into the first tube, 2000pg/ml and mix.
8. Pipette 320 µl of this dilution into the second tube labelled 800 pg/ml and mix.
9. Repeat serial dilutions three more times until 51.2 pg/ml. The standard diluent or cell culture media should be used as the zero standard. These concentrations, 2000 pg/ml, 800 pg/ml, 320 pg/ml, 128 pg/ml, 51.2 pg/ml and 0pg/ml are used to construct the standard curve.

## 8.3. Assay protocol

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. After running the assay, retain the plate frame for the second partial plate.

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 to use, it has been contaminated. DO NOT USE.**

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 2).
3. Remove excess microplate strips from the frame and store in the resealable foil bag.
4. Add 100 µl of standard or sample per well. Cover with adhesive strip provided and incubate for 1 hour at room temperature (20–25°C).
5. Remove adhesive strip and 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 wash bottle, 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.
6. Add 100 µl of biotinylated antibody reagent to each well that is to be used. Cover with the adhesive strip provided and incubate for 2 hours at room temperature (20–25°C).
7. Repeat the aspiration/wash step as in step 5.
8. Add 100 µl of pre-diluted streptavidin-HRP conjugate. Cover with a new adhesive strip and incubate for 30 minutes at room temperature (20–25°C).
9. Repeat the aspiration/wash step as in step 5.

**10.** Add 100 µl of TMB substrate solution into each well and 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.

**11.** Add 100 µl of stop solution to each well.

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

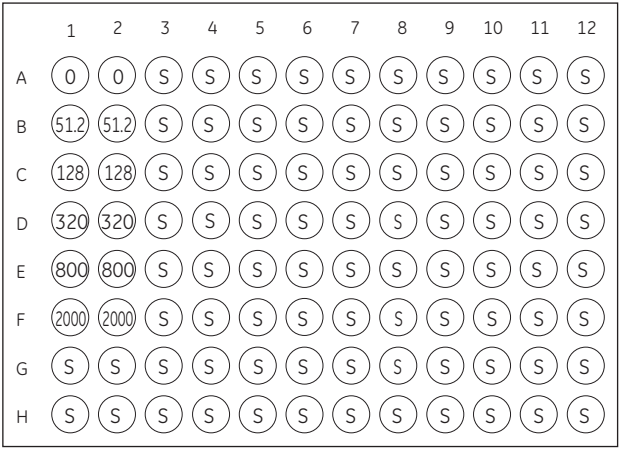


**Figure 2.** Recommended positioning of standards (0–2000 pg/ml) and samples (S).

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

| | Zero standard | Standards | Samples |
|---|---|---|---|
| Standard | – | 100 | – |
| Standard diluent or cell culture media | 100 | – | – |
| Sample | – | – | 100 |
| Cover plate, incubate at room temperature for 1 hour | | | |
| Aspirate/decant and wash vigorously all wells three times with wash buffer | | | |
| Biotinylated antibody reagent | 100 | 100 | 100 |
| 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 for 30 minutes at room temperature | | | |
| 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 | | | |

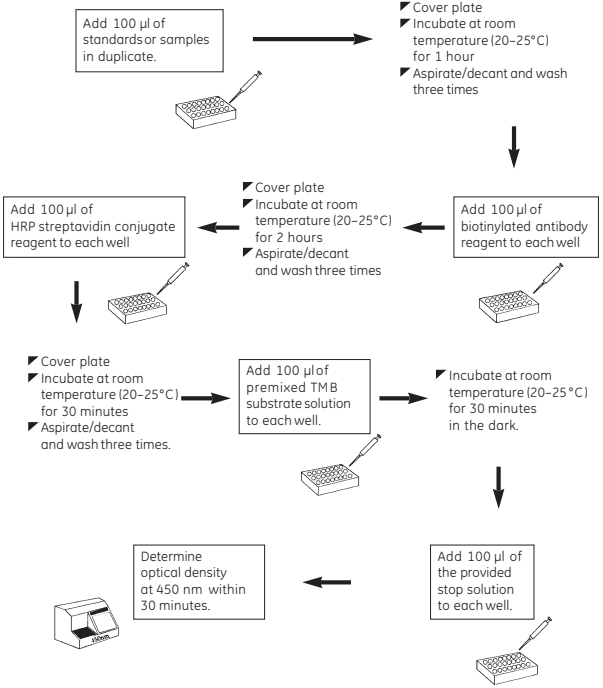


**Figure 3.** Summary of assay protocol

# 9. Data processing

## 9.1. Calculation of results

Typical assay data are shown in table 2.

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 using a log/log plot and regression analysis can be applied to the log transformation (figure 4). 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 | 0.060 | – |
| 51.2 pg/ml standard | 0.145 | 0.085 |
| 128 pg/ml standard | 0.271 | 0.211 |
| 320 pg/ml standard | 0.520 | 0.460 |
| 800 pg/ml standard | 1.044 | 0.984 |
| 2000 pg/ml standard | 2.125 | 2.065 |

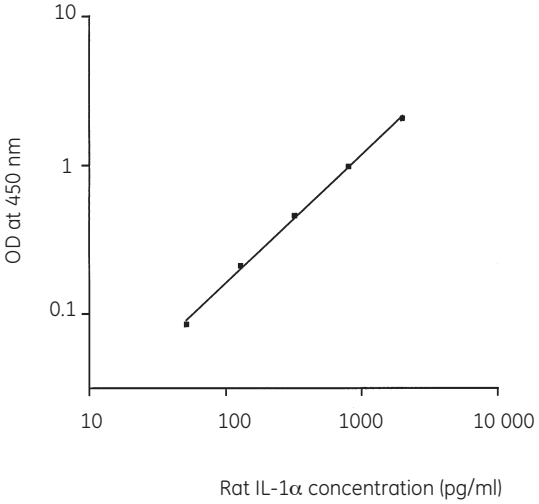


**Figure 4.** Typical rat IL-1α standard curve

# 10. Additional information

## 10.1. Specificity

This ELISA is specific for the measurement of natural and recombinant rat IL-1α. It does not crossreact with rat TNFα, rat IL-1β, rat IL-6, mouse TNFα, mouse IL-1β, human TNFα, human IL-1α, human IL-1β, human IL-1RA. There is minor cross-reactivity (~3%) with mouse IL-1α.

## 10.2. Calibration

The standards in this ELISA are calibrated to an in-house rat IL-1α reference standard. At the time of product release, no NIBSC reference standard was available for rat IL-1α.

## 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 (r)IL-1α was determined to be <15 pg/ml (1.5 pg/well), by adding two standard deviations to the optical density of the zero standard and calculating the corresponding concentration from the standard curve.

## 10.5. Normal levels of rat IL-1α

Serum and plasma samples were collected from apparently healthy rats and run in this assay. The levels of rat IL-1α found in each sample type are reported in the following table:

| Sample type | Average | Range |
|---|---|---|
| Serum samples (n=20) | 27.4 pg/ml | 0–55.9 pg/ml |
| Plasma samples (n=18) | 17.4 pg/ml | 1.3–37.9 pg/ml |

## 10.6. Recovery

Cytokine recovery was determined by adding recombinant rat IL-1α into serum samples from apparently healthy mice. Mean recoveries were as follows:

| Sample type | Spike level | | |
|---|---|---|---|
| | 100 pg/ml | 500 pg/ml | 2000 pg/ml |
| Serum | 92% | 93% | 92% |
| Plasma | 95% | 93% | 93% |

## 10.7. Linearity of dilution

Linearity of dilution was determined by serially diluting four positive sample. The dilutions were run in the ELISA and 'observed' doses plotted against the 'expected' doses. The relationship is given in the equation observed value = 0.959 (expected value) + 22.9, $R^2 = 0.958$.

## 10.8. Troubleshooting guide

**1. Low optical densities**

1. Check the colour of the substrate in the wells. If it is blue add acid to terminate the reaction and develop the correct yellow colour prior to reading.
2. Check reader wavelength.
3. Ensure all reagents have been equilibrated to room temperature before use.
4. Check reagents have been correctly reconstituted.
5. Check reagents have been stored under the recommended conditions.
6. Check incubation times and temperatures.
7. Ensure that the plate is read within 30 minutes of adding the stop reagent.

**2. High optical densities**

1. Check point 4 above.
2. Check point 6 above.

**3. Poor replication**

1. Ensure automatic washers are working correctly, or that each well is completely filled and emptied at every wash step when hand washing.
2. Check pipette calibration.
3. Ensure troughs used with multichannel pipettes are dedicated to individual components.
4. If splashing occurs when using multishot pipettes, lubricate the pipette barrel.
5. Ensure that no cross contamination occurs by using a fresh adhesive plate cover at each incubation stage.

6. Ensure that plates have been carefully placed into the plate reader to avoid splashing.

**4. Standard replication is good, but the standards do not fit the curve**
Check standard dilution procedure.

**5. High non-specific binding**
Check problem 3, point 1.

**6. Brown 'precipitate' in wells**
Under conditions where there are very high levels of enzyme activity in the wells, a brown precipitate will appear on addition of acid. This will initially yield a high optical density but this will decay rapidly. This is indicative of a dilution error with the streptavidin-HRP conjugate.

## 10.9. Background and references

IL-1α is one of a group of three related polypeptide hormones that also includes IL-1β 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.

Rat IL-1α is produced from a 270 amino acid precursor which, although it is biologically active, is generally not secreted. The mature form of IL-1α is a 156 amino acid protein of 17.5 kDa (determined by SDS-PAGE). Numerous cell types produce IL-1α including: astrocytes, B cells, endothelial cells, keratinocytes, kidney epithelial cells, monocytes and myeloid cell lines.

IL-1α 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-1α production include: dexamethasone, prednisolone, 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-1$\alpha$ and IL-1$\beta$ 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 0–30000 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 resorbtion, wound healing, haematopoiesis and inflammatory diseases, including diabetes, periodontitis and rheumatoid arthritis.

IL-1 is considered to be a potent pro-inflammatory cytokine, which together with TNF$\alpha$ is considered to be central in orchestrating the effector phase of immune and inflammatory responses. Dinarello has written a comprehensive recent (1996) review of IL-1 in disease.

**1.** DINARELLO, C.A. *et al., Blood*, **77**, p.1627, 1991.

**2.** CANNON, J.G. *et al., J. Infect. Dis.,* **161**, p.79, 1990.

**3.** GRASSI, J. *et al., J. Immunol. Methods,* **123**, p.193, 1989.

**4.** LONNERMANN, G. *et al., Lymphokine Res.,* **7**, p.75, 1988.

**5.** ZUCALL, J.R. *et al., Blood,* **69**, p.33, 1987.

**6.** DINARELLO, C.A. *et al., J. Clin. Invest.,* **77**, p.1743, 1987.

**7.** DINARELLO, C.A. *et al., Year Immunol.,* **2**, p.68, 1986.

**8.** KRONHEIM, S.R. *et al., J. Exp. Med.,* **181**, p.1490, 1985.

**9.** DINARELLO, C.A., *Blood,* **87**, p.2095, 1996

# 11. Related products

**Biotrak range of human cytokine ELISA systems**

| | |
|---|---|
| Interleukin-1α [(h)IL-1α] | RPN 2750 |
| Interleukin-1β [(h)IL-1β] | RPN 2751 |
| Interleukin-2 [(h)IL-2] | RPN 2752 |
| Interleukin-4 [(h)IL-4] | RPN 2753 |
| Interleukin-5 [(h)IL-5] | RPN 2761 |
| Interleukin-6 [(h)IL-6] | RPN 2754 |
| Interleukin-8 [(h)]IL-8] | RPN 2764 |
| Interleukin-10 [(h)IL-10] | RPN 2755 |
| Interleukin-12 [(h)IL-12], (p40 and p70) | RPN 2765 |
| Interleukin-12 [(h)IL-12], (p70) | RPN 2770 |
| Interleukin-13 [(h)IL-13] | RPN 2766 |
| 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 |
| Transforming growth factor-β1 [(h)TGFβ1] | RPN 2763 |

**Biotrak range of high sensitivity human cytokine ELISA systems**

| | |
|---|---|
| Interleukin-1α [(h)IL-1α] | RPN 2780 |
| Interleukin-1β [(h)IL-1β] | RPN 2781 |
| Interleukin-4 [(h)IL-4] | RPN 2783 |
| Interleukin-6 [(h)IL-6] | RPN 2784 |
| Interleukin-10 [(h)IL-10] | RPN 2785 |
| Granulocyte-macrophage colony stimulating factor [(h)GM-CSF] | RPN 2786 |
| Interferon-gamma [(h)IFNγ] | RPN 2787 |
| Tumour necrosis factor-alpha [(h)TNFα] | RPN 2788 |
| Interferon-alpha [(h)IFNα] | RPN 2789 |

**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-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 2722 |
| Interleukin-12 [(m) IL-12], (p70) | RPN 2723 |
| Granulocyte-macrophage colony stimulating factor [(m)GM-CSF] | RPN 2716 |
| Tumour necrosis factor-α [(m)TNFα] | RPN 2718 |

**Biotrak range of rat cytokine ELISA systems**

| | |
|---|---|
| Interleukin-1β [(r)IL-1β] | RPN 2733 |
| Tumour necrosis factor-α [(r)TNFα] | RPN 2734 |

**Biotrak range of cell adhesion molecule assays**

| | |
|---|---|
| Mouse sICAM-1 ELISA | RPN 2721 |

**Range of unlabelled and radiolabelled growth factors and cytokines**

| | |
|---|---|
| Cell proliferation assay system and reagents | |
| Cell proliferation assay system - version 2 | RPN 250 |
| Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement) | RPN 20 |
| Monoclonal anti-bromodeoxyuridine | RPN 202 |
| Cell proliferation labelling reagent | RPN 201 |

**GE Healthcare offices:**

GE Healthcare Bio-Sciences AB
Björkgatan 30 751 84
Uppsala
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5 D-79111
Freiburg
Germany

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue
P.O. Box 1327
Piscataway
NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1
Hyakunincho Shinjuku-ku
Tokyo 169-0073
Japan

**GE Healthcare regional office contact numbers:**

**Asia Pacific**
Tel: + 85 65 6 275 1830
Fax: +85 65 6 275 1829

**Australasia**
Tel: + 61 2 8820 8299
Fax: +61 2 8820 8200

**Austria**
Tel: 01 /57606 1613
Fax: 01 /57606 1614

**Belgium**
Tel: 0800 73 890
Fax: 02 416 82 06

**Canada**
Tel: 1 800 463 5800
Fax: 1 800 567 1008

**Central, East, & South East Europe**
Tel: +43 1 972720
Fax: +43 1 97272 2750

**Denmark**
Tel: 45 70 25 24 50
Fax: 45 16 24 24

**Eire**
Tel: 1 800 709992
Fax: 0044 1494 542010

**Finland & Baltics**
Tel: +358-(0)9-512 39 40
Fax: +358 (0)9 512 39 439

**France**
Tel: 01 6935 6700
Fax: 01 6941 9677

**Germany**
Tel: 0800 9080 711
Fax: 0800 9080 712

**Greater China**
Tel:+852 2100 6300
Fax:+852 2100 6338

**Italy**
Tel: 02 26001 320
Fax: 02 26001 399

**Japan**
Tel: +81 3 5331 9336
Fax: +81 3 5331 9370

**Korea**
Tel: 82 2 6201 3700
Fax: 82 2 6201 3803

**Latin America**
Tel: +55 11 3933 7300
Fax: + 55 11 3933 7304

**Middle East & Africa**
Tel: +30 210 9600 687
Fax: +30 210 9600 693

**Netherlands**
Tel: 0800 82 82 82 1
Fax: 0800 82 82 82 4

**Norway**
Tel: +47 815 65 777
Fax: 47 815 65 666

**Portugal**
Tel: 21 417 7035
Fax: 21 417 3184

**Russia & other C.I.S. & N.I.S**
Tel: +7 (495) 956 5177
Fax: +7 (495) 956 5176

**Spain**
Tel: 902 11 72 65
Fax: 935 94 49 65

**Sweden**
Tel: 018 612 1900
Fax: 018 612 1910

**Switzerland**
Tel: 0848 8028 10
Fax: 0848 8028 11

**UK**
Tel: 0800 515 313
Fax: 0800 616 927

**USA**
Tel: +1 800 526 3593
Fax: +1 877 295 8102

http://www.gehealthcare.com/lifesciences
GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA
UK

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