

GE Healthcare

Amersham
Interleukin-12
[(h)IL-12](p70), Human,
Biotrak ELISA System

Product Booklet

Code: RPN2770 (96 wells)



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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.

Warning: Contains methanol.

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

Biotinylated antibody reagent, pre-diluted antibody against (h)IL-12 conjugated to biotin, with preservative, 8 ml.

(h)IL-12 standard, 2 vials of baculovirus-derived human recombinant IL-12, lyophilised.

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

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

Standard diluent, with preservative, 12 ml.

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

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

Stop solution, 0.18 M sulphuric acid, 15 ml.

Plate covers, 4 adhesive strips.

4. Description

The Biotrak™ human interleukin-12 (p70) ELISA system from GE Healthcare provides a simple, specific and precise quantitative determination of (h)IL-12(p70) in cell culture supernatants, plasma, serum and urine.

The assay is based on a solid phase ELISA, which utilises an antibody for (h)IL-12 bound to the wells of a microplate together with a biotinylated antibody to (h)IL-12 and streptavidin conjugated to horseradish peroxidase (figure 1). Although GE Healthcare's IL-12 immunoassay contains recombinant IL-12 and antibodies raised against recombinant (h)IL-12 it has been shown to quantitate accurately both natural (h)IL-12 and recombinant (h)IL-12.

(h)IL-12 can be measured in the range 15.4–600 pg/ml (0.77–30 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 <3 pg/ml (0.15 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (h)IL-12
- Small sample size, 50 µl per well

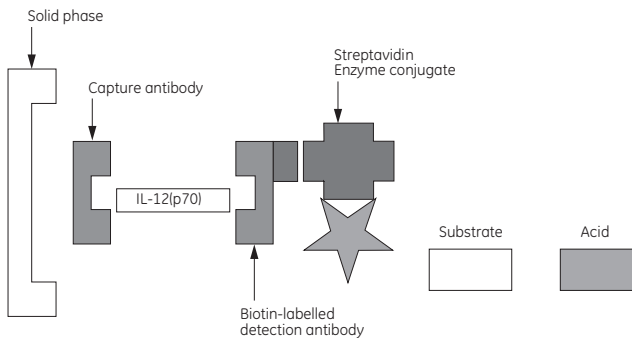


Figure 1. Human IL-12(p70) ELISA assay format

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, 50 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 and equipment for dispensing 12 ml
- Measuring cylinder 2 l
- Distilled or deionised water
- Plate reader capable of reading at 450 nm
- A centrifuge for preparing 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.

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

7. Specimen collection and 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.

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 (h)IL-12 (p70) 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. 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.

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 1ml of streptavidin-HRP buffer per strip being run.

8.2. Preparation of working standards

1. Two vials of lyophilised 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. **When running culture supernatant samples,** reconstitute standard in distilled or deionised 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 5) below for further instructions. If running a partial plate, refer to step 1) above.

4. **If running serum, plasma or urine samples**, reconstitute standard with distilled or deionised 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) above.
5. Label 6 tubes, one for each standard curve point: 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml, 15.4 pg/ml and 0 pg/ml. Then prepare 2.5 fold serial dilutions for the standard curve as follows:
6. Pipette 240 μ l of appropriate diluent [see steps 3) and 4) above] into each tube.
7. Pipette 160 μ l of the reconstituted standard into the first tube, 600 pg/ml and mix.
8. Pipette 160 μ l of this dilution into the second tube labelled 240 pg/ml and mix.
9. Repeat serial dilutions three more times until 15.4 pg/ml. The standard diluent or cell culture media should be used as your zero standard. These concentrations, 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml, 15.4 pg/ml and 0 pg/ml form 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.

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 before 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 50 μ l of biotinylated antibody reagent to each well that is to be used.
5. Add 50 μ l of standard or sample per well. Cover with adhesive strip provided and incubate for 3 hours at room temperature (20–25°C).
6. 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.
7. Add 100 μ l of pre-diluted 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 μ l 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.

10. Add 100 μ l of stop solution to each well.
11. Determine the optical density of each well within 30 minutes, using a spectrophotometer set to 450 nm.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	S	S	S	S	S	S	S	S	S	S
B	154	154	S	S	S	S	S	S	S	S	S	S
C	384	384	S	S	S	S	S	S	S	S	S	S
D	96	96	S	S	S	S	S	S	S	S	S	S
E	240	240	S	S	S	S	S	S	S	S	S	S
F	600	600	S	S	S	S	S	S	S	S	S	S
G	S	S	S	S	S	S	S	S	S	S	S	S
H	S	S	S	S	S	S	S	S	S	S	S	S

Figure 2. Recommended positioning of standards (0–600 pg/ml) and samples (S)

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

	Zero standard	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 3 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			

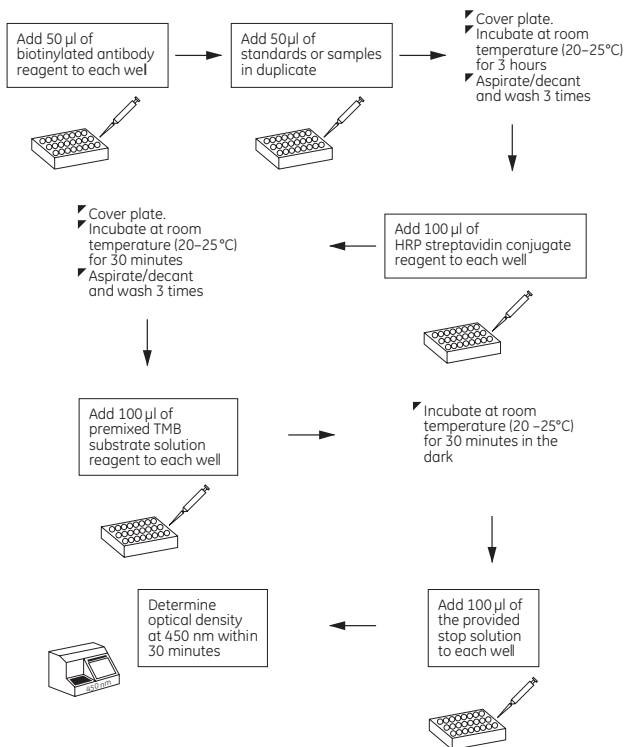


Figure 3. Summary of assay protocol

9. Data processing

9.1. Calculation of results

Typical assay data is 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 linearised using a log/log plot and regression analysis can be applied to the log transformation.

Figure 4 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	0.040	-
15.4 pg/ml standard	0.082	0.042
38.4 pg/ml standard	0.166	0.126
96 pg/ml standard	0.376	0.336
240 pg/ml standard	0.843	0.803
600 pg/ml standard	1.859	1.819

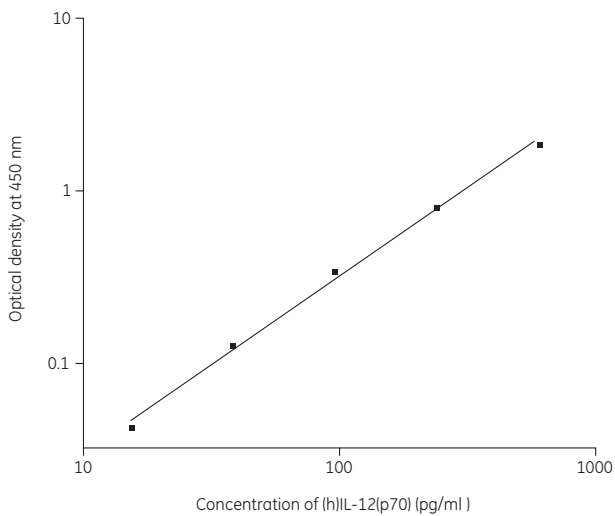


Figure 4. Typical human curve IL-12 (p70)

10. Additional information

10.1. Specificity

This ELISA is specific for the measurement of biologically-active natural and recombinant human IL-12(p70). Recombinant human p40 does not crossreact or interfere with this assay. It does not crossreact with human; IL-1 , IL-1 , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-13, IL-15, TNF , IFN , GM-CSF, GRO , GRO , mouse IL-12(p70) or mouse IL-12 p40 homodimer.

10.2. Calibration

The standards in this ELISA are calibrated to the NIBSC recombinant IL-12 standard 95/544. One (1) pg of Biotrak standard = 1.66 pg of NIBSC standard.

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-12(p70) was determined to be <3 pg/ml (0.15 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 human IL-12 (p70)

Serum, plasma and urine samples were collected from normal human donors and run in this assay. The levels of human IL-12 (p70) found in each sample type are reported below:

Sample type	Average	Range
Serum samples (n=18)	1.20 pg/ml	0–7.9 pg/ml
Plasma samples (n=26)	1.37 pg/ml	0–23.2 pg/ml
Urine samples (n=14)	0.36 pg/ml	0–1.4 pg/ml

10.6. Recovery

Cytokine recovery was determined by spiking various levels of recombinant human IL-12 into normal human serum, plasma, and urine samples and a standard diluent control buffer.

Mean recoveries were as follows:

Sample type	Spike level				
	50 pg/ml	95 pg/ml	200 pg/ml	380 pg/ml	400 pg/ml
Serum (n=8)	-	93%	101%	98%	-
Plasma (n=8)	-	82%	93%	96%	-
Urine	63%	-	-	-	67%

10.7. Linearity of dilution

Linearity of dilution was determined by serially diluting five different positive sample (including plasma, serum, urine and cell culture supernatant). The dilutions were run in the ELISA and 'found' doses plotted against the 'expected' doses.

The relationship is given in the equation

observed value = 1.17 (expected value) + 1.05, $R^2 = 0.993$.

10.8. Effect of human IL-12 (p40)

Up to 100 ng/ml of human p40 has no effect on measured human IL-12 (p70) levels in this assay.

10.9. Troubleshooting guide

Problem: 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.

Problem: High optical densities

1. Check low optical densities point 4.
2. Check low optical densities point 6.

Problem: 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.

Problem: Standard replication is good, but the standards do not fit the curve

Check standard dilution procedure.

Problem: High non-specific binding

Check poor replication point 1

Problem: 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.

10.10. Background and references

IL-12 was initially discovered by its ability to potentiate natural killer cell activity. It is now known to have multiple effects on different lymphocyte populations. In contrast to most cytokines, which possess only one polypeptide chain, IL-12 is a disulphide-linked heterodimer composed of a heavy chain of 40 kDa (p40) and light chain of 35 kDa (p35) encoded by separate genes. The mRNA encoding p35 is constitutively expressed by a variety of cell types, whereas expression of p40 mRNA tends to be more closely correlated with the production of the heterodimer. The heterodimer, termed p70, is considered to be the biologically active IL-12.

Both p40 and a homodimeric form of p40, termed (p40)₂ bind to the human IL-12 receptor. However, the p40 forms do not trigger biological activity and in fact specifically inhibit IL-12 mediated responses. p40 is produced in large excess over IL-12 (p70) and p40 has been proposed to be a natural IL-12 antagonist.

Recent studies have demonstrated that IL-12 is a key cytokine which orchestrates the immune response to invading micro-organisms. Its biological activity has been comprehensively reviewed by Ricardo T. Gazzinelli. In brief, IL-12 is considered to be important in controlling pathogens during the early stages of infection. Macrophages stimulated by microbes release IL-12 which acts upon natural killer (NK) cells to release IFN . IFN is a potent stimulator of macrophage effector functions against invasive pathogens. IL-12 also plays a key role in biasing T-cell differentiation towards the Th1 phenotype. Murine Th1 lymphocytes exclusively producing IL-2, IFN and TNF are responsible for specific cell-mediated immunity. In contrast, murine Th2 cells exclusively produce IL-4, IL-5, IL-10 and IL-13 and mediate humoral immunity. Successful resolution of many infectious diseases is dependent upon the appropriate induction of a Th1 or Th2 response. The use of IL-12, or IL-12 antagonists in human disease is likely to be an extremely interesting and valuable scientific research field.

In addition to this human IL-12(p70) kit, GE Healthcare also supplies a kit for measuring total human IL-12(p40-p70). Together these kits provide the means to measure biologically active IL-12 (p70) and its natural antagonist IL-12 (p40) in the same sample. Both measurements are likely to be required for a full understanding at the physiological and pathological role of IL-12.

1. TREMBLEAU, S. *et al.*, *Immunology Today*, **16**, pp.383-386, 1995.
2. LAMONT, A.G. and ADORINI, L., *Immunology Today*, **17**, pp.214-217, 1996.
3. GAZZINELLI, R.T., *Molecular Medicine Today*, **2**, pp.225-267, 1996.

10.11. Related products

Biotrak range of human cytokine ELISA systems

Interleukin-1 [(h)IL-1]	RPN 2750
Interleukin-1 [(h)IL-1]	RPN 2751
Interleukin-2 [(h)IL-2]	RPN 2752
Soluble interleukin-2 receptor [(h)sIL-2R]	RPN 2767
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-13 [(h)IL-13]	RPN 2766
Granulocyte-macrophage colony stimulating factor [(h)GM-CSF]	RPN 2756
Interferon-gamma [(h)IFN]	RPN 2757
Tumour necrosis factor-alpha [(h)TNF]	RPN 2758
Interferon-alpha [(h)IFN]	RPN 2759
Transforming growth factor- ₁ [(h)TGF ₁]	RPN 2763

Biotrak range of high sensitivity human cytokine ELISA systems

Interleukin-1 [(h)IL-1]	RPN 2780
Interleukin-1 [(h)IL-1]	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)IFN]	RPN 2787
Tumour necrosis factor-alpha [(h)TNF]	RPN 2788
Interferon-alpha [(h)IFN]	RPN 2789

Biotrak range of mouse cytokine ELISA systems

Interleukin-1 [(m)IL-1]	RPN 2719
Interleukin-1 [(m)IL-1]	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 2722
Granulocyte-macrophage colony stimulating factor [(m)GM-CSF]	RPN 2716
Interferon-gamma [(m)IFN]	RPN 2727
Tumour necrosis factor- [(m)TNF]	RPN 2718

Biotrak range of rat cytokine ELISA systems

Interleukin-1 [(r)IL-1]	RPN2733
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Biotrak range of cell adhesion molecule assays

h-sICAM-1 ELISA	RPN 247
h-sELAM-1 ELISA	RPN 248
h-sVCAM-1 ELISA	RPN 249
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

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