GE Healthcare

# Amersham

# High Sensitivity Granulocyte-Macrophage Colony Stimulating Factor [(h)GM-CSF] human, Biotrak ELISA system

96 wells

## Product Booklet

Code: RPN2786

# 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°C to 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. Description

The Biotrak™ high sensitivity human granulocyte-macrophage colony stimulating factor ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (h) GM-CSF in cell culture supernatants, plasma, serum and urine.

The assay system is based on a solid phase ELISA, which utilizes an antibody for (h) GM-CSF bound to the wells of a microplate together with a biotinylated antibody to (h) GM-CSF and Amdex™ amplification reagent. Although GE Healthcare's (h) GM-CSF immunoassay contains recombinant (h) GM-CSF and antibodies raised against recombinant (h) GM-CSF, it has been shown to quantitate accurately both natural (h) GM-CSF and recombinant (h) GM-CSF.

(h) GM-CSF can be measured in the range 0.63–20 pg/ml (0.032–1 pg/well) in less than 24 hours using the protocol provided with the kit. Each pack contains sufficient material for 96 wells. If one standard curve is constructed, 41 unknowns can be measured in duplicate.

- High sensitivity – 0.1 pg/ml
- Overnight protocol
- Pre-coated plate
- Specific for (h) GM-CSF

# 4. Introduction

Human granulocyte-macrophage colony stimulating factor [(h) GM-CSF] is a member of the colony stimulating factor family, a group of functionally related cytokines that also includes IL-3, granulocyte-CS (G-CS) and macrophage-CS (M-CS). The CSFs were initially identified by their ability to support the clonal growth of haematopoietic stem cells in semi-solid culture media.

Human GM-CSF is an 18-22 kDa glycoprotein produced by T cells, endothelial cells, fibroblasts and macrophages and in most systems requires an inductive signal. Human GM-CSF is approximately 56% homologous to mouse GM-CSF at the amino acid level, however, human GM-CSF does not bind the mouse GM-CSF receptor and vice-versa. GM-CSF specifically; induces the differentiation of both monocytes and granulocytes from bipotential stem cells, induces the production of IL-1$\alpha$ and TNF by monocytes, functions as a survival and growth factor for eosinophils, granulocytes and macrophages and primes macrophages and polymorphonuclear cells from phagocytosis, superoxide production and arachidonic acid synthesis.

The biological activities of human GM-CSF are mediated through interactions with a specific cell surface receptor expressed by a wide variety of cell types. The human GM-CSF receptor is a heterodimeric molecule comprised of a 85 kDa$\alpha$ and 130 kDa$\beta$ chains. By itself the GM-CSFR$\alpha$ is a low affinity receptor for GM-CSF without signal transduction capabilities. When combined with the $\beta$ subunit the complex possesses a high binding affinity (10-100 pM) and signal transduction. The GM-CSFR$\beta$ is also the $\beta$ subunit of the IL-3R and the IL-5R.

As a differentiation, growth and survival factor for granulocytes GM-CSF is a promising therapy for neutropenia.
Specifically, GM-CSF has applications in a variety of neutropenic conditions including bone marrow dysfunction, infectious disease

and chemotherapy. Treatment of human and other primates with GM-CSF has been shown to increase the number of circulating leukocytes and platelets.

# 5. Summary of the assay

This assay employs the quantitative 'sandwich' enzyme immunoassay technique. An antibody specific for (h) GM-CSF has been coated on the microplate provided in the kit. Samples are pipetted into the wells and the (h) GM-CSF, if present, is bound by the immobilized antibody. A biotinylated antibody reagent is added to the wells and allowed to bind to any (h) GM-CSF bound by the immobilized antibody in the first incubation. After washing away any unbound biotinylated antibody an Amdex amplification reagent is added, any (h) GM-CSF which was bound by both the immobilized and the biotinylated antibody will be bound by the amplification reagent. Following a wash to remove any unbound amplification reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of (h) GM-CSF bound in the initial step.

The Amdex amplification reagent is a high performance conjugate based on a unique chemistry that utilizes a hydrophilic straight chain dextran backbone to which many hundreds of horseradish peroxidase molecules are covalently coupled, together with, on average, ten streptavidin molecules. The result is a multifunctional conjugate with a significantly enhanced activity and with well controlled non-specific binding properties.

In addition to the samples to be tested, a series of wells is prepared using known concentrations of the human GM-CSF 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) GM-CSF in the unknown samples is then determined.

# 6. 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)GM-CSF microplate** – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against (h) GM-CSF.

**Biotinylated antibody reagent** – antibody against (h) GM-CSF conjugated to biotin, with preservative, 8 ml.

**(h)GM-CSF standard** – 2 vials of recombinant human GM-CSF, lyophilized.

**Amdex amplification reagent** – lyophilized.

**Standard diluent** – with preservative, 12 ml, 2 vials.

**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, 15 ml.

**Plate covers** – 6 adhesive strips.

# 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 (20 ml, 50 ml, 100 ml, 500ml, 1.00 ml and 5.00 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
Plate shaker

**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).

## 7.2. 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 activity. Avoid freezing and thawing samples more than once. Test samples should be assayed in duplicate each time the ELISA is performed, 50 ml of sample per well is required in this assay.

The measurement of cytokines in serum and plasma has been reported to be affected by non-specific matrix effects which may

vary between samples from different individuals (1–3). Dilution of such samples in the diluent supplied may help to reduce these interference effects.

**Dilution of test samples**
If it is suspected that the (h) GM-CSF 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.

It remains the investigators responsibility to validate the chosen sample dilution.

## 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. It is important that all the wells are washed thoroughly and uni formly. If using an automatic washer, check operation of heads before starting. If washing by hand ensure wells are completely filled at each wash.

**10.** Timings in this assay are critical and should be adhered to strictly. Failure to do so could alter optical densities significantly.

**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 the contents of the bottle to 1500 ml using distilled water. Store at 2–8°C until the expiry date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

**Amdex amplification reagent**

Reconstitute the Amdex reagent in 11 ml of distilled or deionized water approximately 15 minutes before use. Reconstituted reagent may be stored at -15°C to -30°C for up to 1 week.

**(h)GM-CSF standard**

Reconstitute 1 vial of (h) GM-CSF standard with distilled or deionized water. Reconstitution volume is shown on the vial label. Mix by gently inverting the vial.

It is important that the diluent selected for dilution of the standard reflects the environment of the samples being measured. Standard diluent will be suitable for serum, plasma or urine measurements. If your samples are cell culture supernatants, the culture media will be suitable for preparation of the standard curve.

Testing of RPMI with different lots and concentrations of foetal bovine serum has shown that this ELISA is not adversely affected by culture medium. Therefore when culture supernatants, serum and plasma samples are assayed on the same plate, standards prepared in standard diluent may be used.

**Preparation of working standards**

**1.** Label 6 polypropylene tubes, 0.63, 1.25, 2.5, 5, 10 and 20 pg/ml.

**2.** Pipette 1.48 ml of standard diluent into the 20 pg/ml tube.

**3**. Pipette 500 µl of standard diluent into all remaining tubes.

**4.** Into the 20 pg/ml tube pipette 20 µl of reconstituted (h)GM-CSF standard and mix thoroughly.

**5.** Transfer 500 µl from the 20 pg/ml tube to the 10 pg/ml tube and mix thoroughly.

**6.** Repeat this doubling dilution successively with the remaining tubes.

**7.** 50 µl aliquots from each serial dilution will give rise to 6 standard levels of (h) GM-CSF ranging from 0.63 to 20 pg/ml.

**NOTE:** Working standards should be freshly prepared before each assay, and not re-used.

**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. Pipette 50 µl of standard diluent or cell culture medium (see section on reagent preparation) into NSB wells.
5. Pipette 50 µl of standard into the appropriate wells.
6. Pipette 50 µl of sample into the appropriate wells.
7. Pipette 50 µl of the biotinylated antibody reagent into all wells. Cover with the adhesive strip provided and incubate overnight (16–18 hours) at room temperature (20–25°C).
8. Aspirate or decant each well and wash, repeating the process three times for a total of four washes. Wash thoroughly by completely filling each well with wash buffer using a washbottle, 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.
9. Pipette 100 µl of Amdex amplification reagent into all wells. Cover with a new adhesive strip and incubate for 1 hour at room temperature (20–25°C) **with continuous shaking**.
10. Repeat the aspiration/wash step as in step 8.
11. Pipette 100 µl of TMB substrate solution into all wells, incubate for 1 hour at room temperature (20–25°C) **with continuous shaking**. If the substrate reagent is bright blue prior to use, do not use. Do not cover the plate with aluminium foil or an adhesive strip.

**12.** Pipette 100 µl of stop solution into all wells.

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

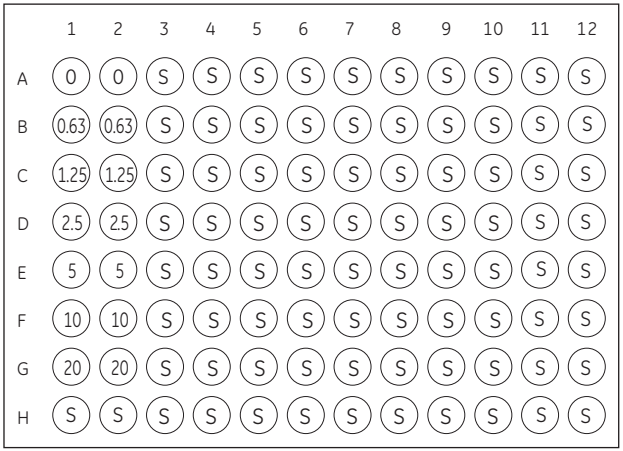


**Figure 1**. Recommended positioning of standard (0.63–20 pg/ml) and sample wells (S).

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

| | Zero standard | Standards | Samples |
|---|---|---|---|
| Standard | – | 50 | – |
| Standard diluent or cell culture media* | 50 | – | – |
| Sample | – | – | 50 |
| Biotinylated antibody reagent | 50 | 50 | 50 |
| Cover plate, incubate at room temperature (20–25°C) overnight (16–18 hours). | | | |
| Aspirate/decant and wash thoroughly all wells four times with wash buffer | | | |
| Amdex amplification reagent | 100 | 100 | 100 |
| Cover plate, incubate at room temperature (20–25°C) on a plate shaker for 1 hour. | | | |
| Aspirate/decant and wash thoroughly all wells four times with wash buffer | | | |
| Substrate | 100 | 100 | 100 |
| Incubate at room temperature (20–25°C) on a plate shaker for 1 hour | | | |
| Stop solution | 100 | 100 | 100 |
| Determine optical density at 450 nm within 30 minutes. | | | |

* Use 50 µl of cell culture media if your standard curve is diluted in cell culture media. See section on reagent preparation.

## 7.5. Calculation of results

Average the duplicate readings for each standard 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 can 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 41 unknowns in duplicate.

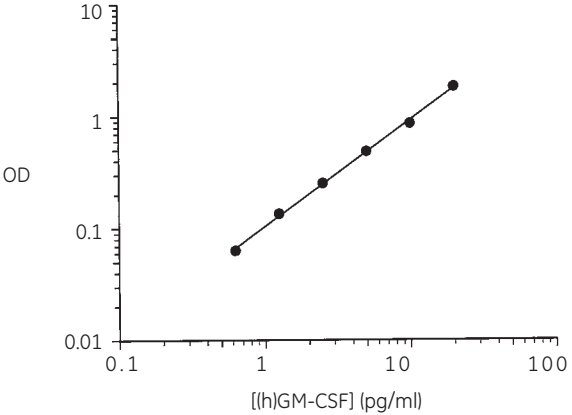


**Figure 2**. Standard curve

**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 | Zero density |
|---|---|---|
| standard subtracted | | |
| Zero standard | 0.090 | – |
| 0.63 pg/ml standard | 0.154 | 0.064 |
| 1.25 pg/ml standard | 0.225 | 0.135 |
| 2.5 pg/ml standard | 0.342 | 0.252 |
| 5 pg/ml standard | 0.572 | 0.482 |
| 10 pg/ml standard | 0.945 | 0.855 |
| 20 pg/ml standard | 1.903 | 1.813 |

When running these high sensitivity assays some variation in OD values may be observed. However control and sample values will not be affected.

# 8. Additional information

## 8.1. Specificity

This assay recognizes both natural and recombinant (h)GM-CSF. It does not cross react with human IL-1$\alpha$, IL-1$\beta$, IL-2, IL-3, IL-6, IL-7, IL-8, TNF$\alpha$, IFN$\gamma$, or mouse GM-CSF.

## 8.2. Calibration

The standards in this ELISA are calibrated to the NIBSC reference lot 88/646.
1 pg of GE Healthcare standard = 1 pg of NIBSC standard
= 0.01 NIBSC unit.

## 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%.

**Precision profile**

A precision profile was generated by preparing replicates of each of the standards and calculating the standard deviation and coefficient of variation at each concentration.

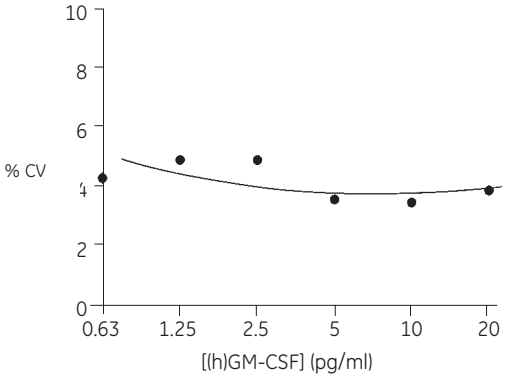


**Figure 3.** Precision profile

## 8.4. Sensitivity

The minimum detectable dose of (h) GM-CSF was determined to be 0.1 pg/ml (0.005 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. Parallelism

The linearity of dilution was determined by serially diluting six different positive samples. The dilutions were run in the ELISA and 'observed' doses were plotted against 'expected' doses.

## 8.6. Expected values

The values found in normal individuals are shown below:-

| **Sample** | **Average value** | **Range** |
|---|---|---|
| Serum (n=21) | 0.53 pg/ml | 0–7.7 pg/ml |
| Plasma (n=33) | 2.5 pg/ml | 0–48 pg/ml |
| Urine (n=5) | 0.48 pg/ml | 0–1.1 pg/ml |

# 9. References


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# 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-2 [(h)IL-2] | RPN 2752 |
| 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 |
| Interleukin-5 [(h)IL-5] | RPN 2761 |
| Granulocyte colony stimulating factor [(h)G-CSF] | RPN 2762 |
| Transforming growth factor $\beta_1$ [(h)TGF$\beta$]$_1$ | RPN 2763 |

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

| | |
|---|---|
| Interleukin-1a [(h)IL-1α] | RPN 2780 |
| Interleukin-1b [(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 |
| 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-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 |
| Tumour necrosis factor-alpha [(m) TNFα] | RPN 2718 |
| sICAM-1 | RPN 2721 |

**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**

**Pipettes**

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 µl | 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 4 and 12 multi-channel variable volume pipettes, a range of pipette tips and related accessories.

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