GE Healthcare

Amersham Granulocyte-Macrophage Colony Stimulating Factor [(m)GM-CSF] Mouse, Biotrak ELISA System

96 wells

Product Booklet

Code: RPN2716

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 2–8ºC.

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™ mouse granulocyte-macrophage colony stimulating factor ELISA system from GE Healthcare provides a simple, specific, reliable and precise quantitative determination of (m)GM-CSF in cell culture supernatants and serum.

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

(m)GM-CSF can be measured in the range 10–250 pg/ml (0.5–12.5 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 – 5 pg/ml (0.25 pg/well)
- Same day protocol
- Pre-coated microplate
- Specific for (m)GM-CSF

4. Introduction

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

Mouse GM-CSF is an 18-22 kDa glycoprotein produced by T cells, fibroblasts and macrophages in response to bacterial endotoxin, mitogenic lectins and inflammatory cytokines such as IL-1 and TNF. Mouse GM-CSF is approximately 56% homologous to human GM-CSF at the amino acid level; however, mouse GM-CSF does not bind the human 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-1α and TNF by monocytes; functions as a survival and growth factor for eosinophils, granulocytes and macrophages; and primes macrophages and polymorphonuclear cells for phagocytosis, superoxide production and arachidonic acid synthesis.

The biological activities of mouse GM-CSF are mediated through interactions with a specific cell surface receptor expressed by a wide variety of cell types. The mouse GM-CSF receptor is a heterodimeric molecule comprised of 85 kDa and 130 kDb chains. By itself, GM-CSFR is a low affinity (~1 nM) receptor for GM-CSF without signal transduction capabilities. GM-CSFR b by itself is not a ligand for GM-CSF but confers high affinity binding (10-100 pM) and signal transduction upon the α/β heterodimer. GM-CSFR β is also the β subunit of the IL-3R and IL-5R.

As a differentiation, growth and survival factor for granulocytes, GM-CSF is a promising therapy for various states of 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 (m)GM-CSF has been coated on to the microplate provided in the kit. Samples are pipetted into the wells, and the (m)GM-CSF, if present, is bound by the immobilized antibody. After washing away any unbound sample proteins, an enzyme-linked antibody specific for (m)GM-CSF is added to the wells and allowed to bind to any (m)GM-CSF 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)GM-CSF 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)GM-CSF standard. A curve, plotting the optical density versus the concentration of (m)GM-CSF in these standard wells, is prepared. By comparing the optical density of the samples to this standard curve, the concentration of the (m)GM-CSF in the unknown samples is then determined.

6. Contents 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)GM-CSF microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse GM-CSF.

(m)GM-CSF conjugate - pre-diluted antibody against mouse GM-CSF conjugated to horseradish peroxidase, with preservative, 12 ml.

(m)GM-CSF standard - pre-diluted recombinant mouse GM-CSF, lyophilized, 2 vials.

Plate reagent - with preservative, 8 ml.

Standard diluent - with preservative, 12 ml.

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

Pre-mixed TMB substrate solution - substrate solution, with preservative, 12 ml.

Stop solution - 0.18 M sulphuric acid.

Plate covers - adhesive strips, 4.

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 (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

Optional equipment

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

7.2. Sample preparation

Cell culture supernatants

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

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

Serum

Serum samples should be allowed to clot at room temperature. Immediately after clotting, spin down. Specimens should be clear and non-haemolyzed 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 2-8ºC. Specimens to be stored for longer periods of time should be frozen at -70ºC to avoid loss of biologically active cytokine. Avoid freezing and thawing samples more than once.

Dilution of test samples
If you suspect that the (m)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.

7.3. Procedural notes

1) 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) For sample and conjugate incubations a humidified incubator may be used to help prevent evaporation loss due to incomplete plate sealing.

8. Assay procedure

8.1. 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 2–8ºC until the expiy date of the kit. Do not use wash buffer if it becomes visibly contaminated on storage.

(m)GM-CSF 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. If your samples are cell culture supernatants, the culture media will be suitable for preparation of the standard curve.

Reconstitute the (m)GM-CSF 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 250 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 (10-250 pg/ml). Use standards within 15 minutes of dilution.

The reconstituted 250 pg/ml (m)GM-CSF 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: 50 pg/ml and 10 pg/ml. Pipette 400 µl of appropriate diluent into each tube. Pipette 100 µl of reconstituted (m)GM-CSF standard into the first tube labelled 50 pg/ml and mix. Pipette 100 µl of this dilution into the second tube labelled 50 pg/ml and mix. These concentrations, 250 pg/ml, 50 pg/ml and 10 pg/ml provide the standard curve points.

8.2. 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 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.**

8.3. 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 pre-diluted (m)GM-CSF conjugate. Cover with a new adhesive strip and incubate for 1 hour at 37ºC ± 2ºC.

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.

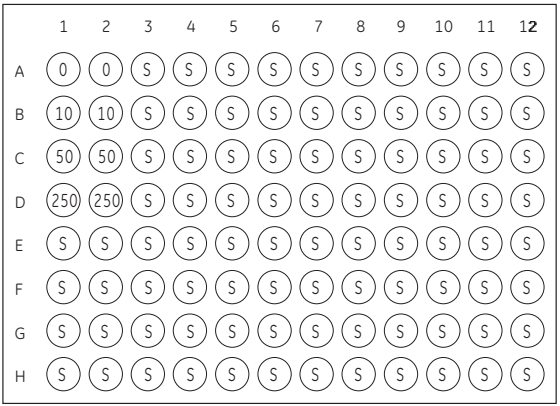

Figure 1. Recommended positioning of standard (0–250 pg/ml) and sample wells (S).

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 37ºC ± 2ºC 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 37ºC ± 2ºC 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.			

9. 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.

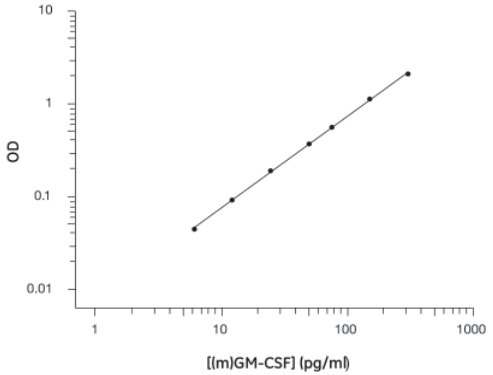

Figure 2. Standard curve

9.1. Typical assay data

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	0.031	–
6.25 pg/ml standard	0.075	0.044
12.5 pg/ml standard	0.121	0.090
25 pg/ml standard	0.215	0.184
50 pg/ml standard	0.388	0.357
75 pg/ml standard	0.571	0.540
150 pg/ml standard	1.116	1.085
300 pg/ml standard	2.061	2.030

10. Additional Information

10.1. Specificity

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

10.2. 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%.

10.3. Sensitivity

The minimum detectable dose of biologically active (m)GM-CSF was determined to be 5 pg/ml (0.25 pg/well), by adding two standard deviations to the optical density value of zero and calculating the corresponding concentration from the standard curve.

10.4. Recovery

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

Standard diluent spike	Serum value	Recovery
127 pg/ml	74 pg/ml	58%
67 pg/ml	38 pg/ml	57%
33 pg/ml	21 pg/ml	64%
16 pg/ml	14 pg/ml	85%

11. References

1. VANNUCCHI, A.M. *et al., Blood,* **76**, p.1473, 1990.

2. KREIDER, B.L. *et al., Mol. Cell Biology,* **10**, p.4846, 1990.

3. ULICH, T.R. *et al., Am. J. Pathology,* **137,** p.369, 1990.

4. KEITH, W.N. *et al., Brit. J. Cancer,* **62**, p.388, 1990.

5. GOUGH, N.M. *et al., Nature,* **309,** p.763, 1984.

6. WILLIAMS, N. *et al., J. Cellular Physiology,* **110,** p.101, 1982.

7. DEXTER, T.M. *et al., J. Exp. Med.*, **152**, p.1036-1047, 1980.

12. Related products

Human cytokine ELISA systems from the Biotrak assay range

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	**Code**
0.5–10 µl	RPN 2340
5–50 µl	RPN 2341
50–200 µl	RPN 2342
200–1000 µl	RPN 2343
1–5 ml	RPN 2344

Multi-channel, variable volume pipettes

8 channel, 5.0–50 µl	RPN 2372
8 channel, 50–250 µl	RPN 2373

GE Healthcare range also includes fixed volume, 4 and 12 multi-channel variable volume pipettes, a range of pipette tips and related accessories.

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