GE Healthcare

Amersham Granulocyte-Macrophage Colony Stimulating Factor Human [(h)GM-CSF] Biotrak ELISA System

96 wells

Product Booklet

Code: RPN2756

Page finder

1. Legal

http://www.gehealthcare.com/lifesciences

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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 2–8°C.

2.2. Expiry

The expiry date is stated on the package and will normally be at least 4 weeks from the date of despatch

3. 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 human GM-CSF.

Biotinylated antibody reagent - pre-diluted antibody against human GM-CSF conjugated to biotin, with preservative, 8 ml.

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

Streptavidin-HRP concentrate - 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 - substrate reagent, 12–13 ml.

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

Plate covers - 4 adhesive strips.

4. Description

The Biotrak™ 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, serum, plasma 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 microtitre plate together with a biotinylated antibody to (h)GM-CSF and streptavidin conjugated to horseradish peroxidase. Although GE Healthcare (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 15–600 pg/ml (0.75–30 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, 42 unknowns can be measured in duplicate.

- High sensitivity - <2 pg/ml (0.1 pg/well)
- Same day protocol
- Pre-coated plate
- Specific for (h)GM-CSF

5. 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 hematopoietic 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-1α 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 kDaα and 130 kDaβ chains. By itself the GM-CSFRa is a low affinity receptor for GM-CSF without signal transduction capabilities. When combined with the β subunit the complex possesses a high binding affinity (10–100 pM) and signal transduction. The GM-CSFRβ is also the β 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.

6. 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 microtitre plate provided in the kit. Samples are pipetted into the wells along with biotinylated antibody reagent. If present, the (h)GM-CSF is bound by the immobilized antibody and the biotinylated antibody. After washing away any unbound sample proteins and biotinylated antibody, a streptavidin-HRP conjugate is added to the wells. Any (h)GM-CSF 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 unbound conjugate, a substrate solution is added to the wells and color develops in proportion to the amount of (h)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 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.

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
A centrifuge for preparing streptavidin-HRP solution

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

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 biologically active cytokine. Avoid freezing and thawing samples more than once.

Dilution of test samples
If you suspect 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.

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 the wells are washed thoroughly and uniformly. If using an automatic washer check operation of heads before starting. If washing by hand ensure that all wells are completely filled at each wash.

10. 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 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.

Preparation of working standards

a. Two vials of lyophilized standards are provided with this kit. Reconstitute and use one vial per partial plate.

b. Prepare standards shortly before use. Use standards within 15 minutes of dilution.
Do not store reconstituted standards.

c. When **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 your culture medium to prepare the dilutions of the standard curve, go to step e) below for further instructions. If running a partial plate, refer to step a) above.

d. If **running serum or plasma samples**, reconstitute standard with distilled or deionized water. Reconstitution volume is stated on the standard vial label. 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 a) above.

e. 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 1:2.5 serial dilutions for the standard curve as follows:

f. Pipette 240 µl of appropriate diluent (see steps c) and d) above) into each tube.

g. Pipette 160 µl of the reconstituted standard into the first tube, 600 pg/ml and mix.

h. Pipette 160 µl of this dilution into the second tube labelled 240 pg/ml and mix.

i. Repeat serial dilutions three more times. These concentrations, 600 pg/ml, 240 pg/ml, 96 pg/ml, 38.4 pg/ml, 15.4 pg/ml and 0 pg/ml are your standard curve.

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

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. Add 50 µl of biotinylated antibody reagent to each well that is to be used.
5. Add 50 µl of diluted standard or sample per well. Cover with adhesive strip provided and incubate for 3 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 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 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 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.

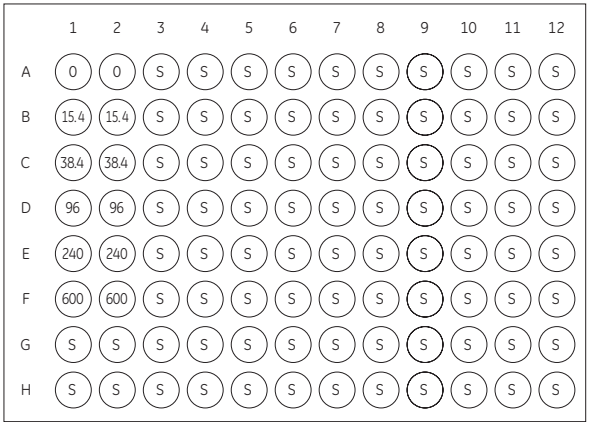
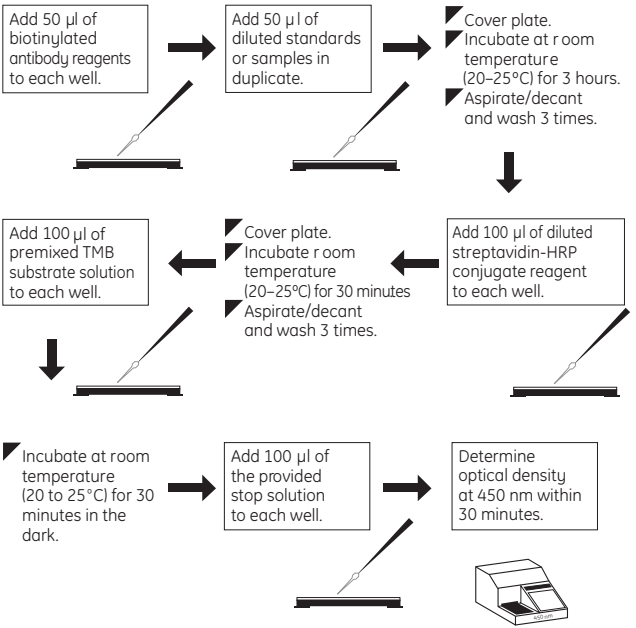


Figure 1. Recommended positioning of standard (0–600 pg/ml) and sample wells (S).

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

	Zero Standard (B_0)	Standards	Samples
Biotinylated antibody	50	50	50
Standard	–	50	–
Sample 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 at room temperature for 30 minutes.			
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.			

Summary of assay protocol



7.5. 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. The protocol describes the generation of 6 points (zero included) by dilution of a stock standard allowing for the measurement of 42 unknowns in duplicate.

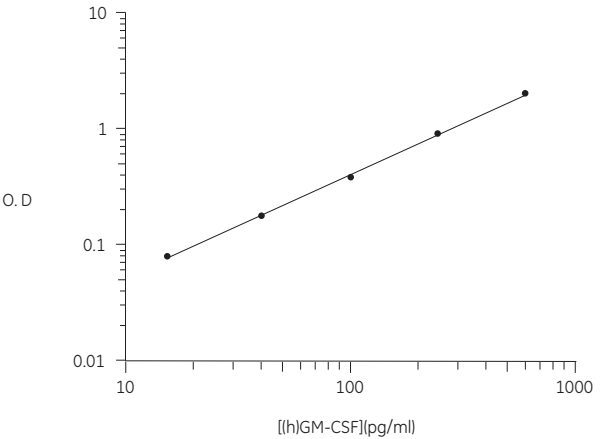


Figure 2. Standard curve

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 density	Zero standard subtracted
Zero standard	0.027	-
15.4 pg/ml standard	0.105	0.078
38.4 pg/ml standard	0.197	0.17
96 pg/ml standard	0.386	0.359
240 pg/ml standard	0.875	0.848
600 pg/ml standard	1.944	1.917

8. Additional information

8.1. Specificity

This assay recognizes both natural and recombinant (h)GM-CSF. It does not cross-react with human IL-1α, IL-1β, IL-2, IL-3, IL-6, IL-7, IL-8, IFNγ, TNFα, or mouse GM-CSF.

8.2. Calibration

The standard in this ELISA is calibrated to NIBSC reference standard 88/646.
1 pg of GE standard = 1 pg of NIBSC standard = 0.01 NIBSC units.

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%.

8.4. Sensitivity

The minimum detectable dose of biologically active (h)GM-CSF was determined to be <2 pg/ml (0.1 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. Expected values

Levels of GM-CSF in healthy individuals are shown below.

	Average	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-6 [(h)IL-6]	RPN 2754
Interleukin-10 [(h)IL-10]	RPN 2755
Interferon-gamma [(h)IFNγ]	RPN 2757
Tumour necrosis factor, alpha [(h)TNFα]	RPN 2758
Interferon-alpha [(h)IFNα]	RPN 2759

Biotrak range of mouse cytokine ELISA systems

Interleukin-1β [(m)IL-1β]	RPN 2720
Interleukin-4 [(m)IL-4]	RPN 2712
Tumour necrosis factor-α [(m)TNF-α]	RPN 2718

Range of unlabelled and radiolabelled growth factors and cytokines

Cell proliferation assay system and reagents

Cell proliferation kit (for immunocytochemical/ immunohistochemical measurement)	RPN 20
Monoclonal anti-bromodeoxyuridine	RPN 202
Cell proliferation labelling reagent	RPN 201

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