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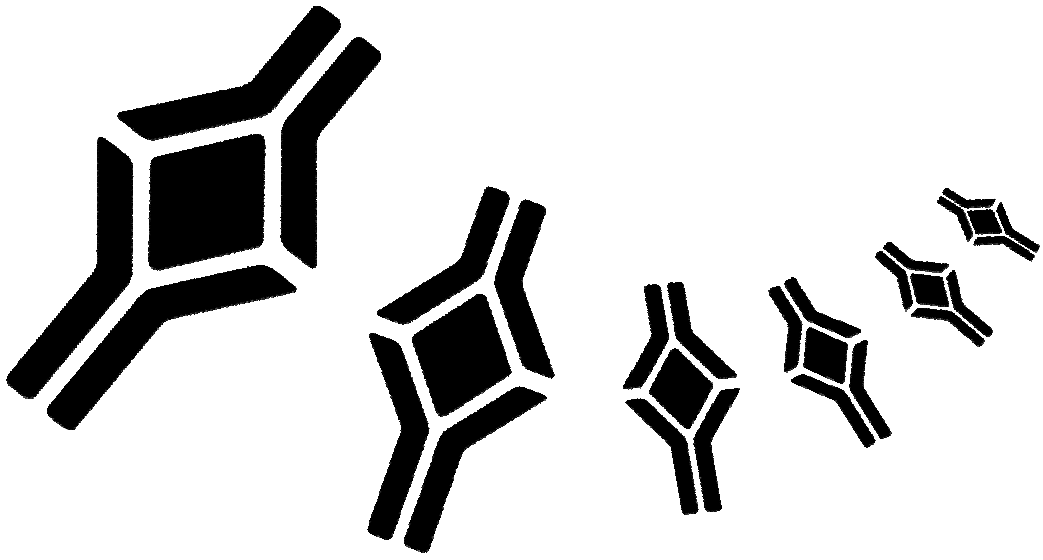


# MOUSE GM-CSF ELISA

Product Data Sheet

Cat. No.: RBMS612R

For Research Use Only



VERSION 51 090708

## CONTENTS



» **This kit is manufactured by:**
**BioVendor – Laboratorní medicína, a.s.**

» **Use only the current version of Product Data Sheet enclosed with the kit!**

# 1 INTENDED USE

The mGM-CSF ELISA is an enzyme-linked immunosorbent assay for quantitative detection of murine GM-CSF in cell culture supernatants, murine serum, plasma or other body fluids. **The mGM-CSF ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

# 2 SUMMARY

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that has been characterized as a molecule capable of stimulating proliferation and differentiation of granulocyte/macrophage progenitor cells as well as modulating the functional activity of mature granulocytes and macrophages. In combination with erythropoietin, it supports the development of erythroid progenitors in vitro. The biological activities of GM-CSF substantially overlap with the effects of Interleukin 3. The reason for the redundancy of these pleiotropic cytokines may at least in part be explained by their competition for binding to common receptors.
GM-CSF is produced by a number of different cell types such as activated T-cells, B-cells, macrophages, mast cells, endothelial cells, fibroblasts, synoviocytes and various tumor cells in response to various stimuli.
Mouse GM-CSF as well as its receptors have been cloned. The encoded glycoprotein consists of 141 amino acid residues and has a molecular weight of about 23 kDa. The murine protein binds to a high affinity receptor complex consisting of two membrane glycoproteins, the GM-CSF receptor $\alpha$ subunit with notable homology to the human receptor and the $\beta$ subunit which also forms part of the receptors for IL-3 and IL-5.
Elevated levels of GM-CSF are found in mice after endotoxin injection.

# 3 PRINCIPLES OF THE TEST

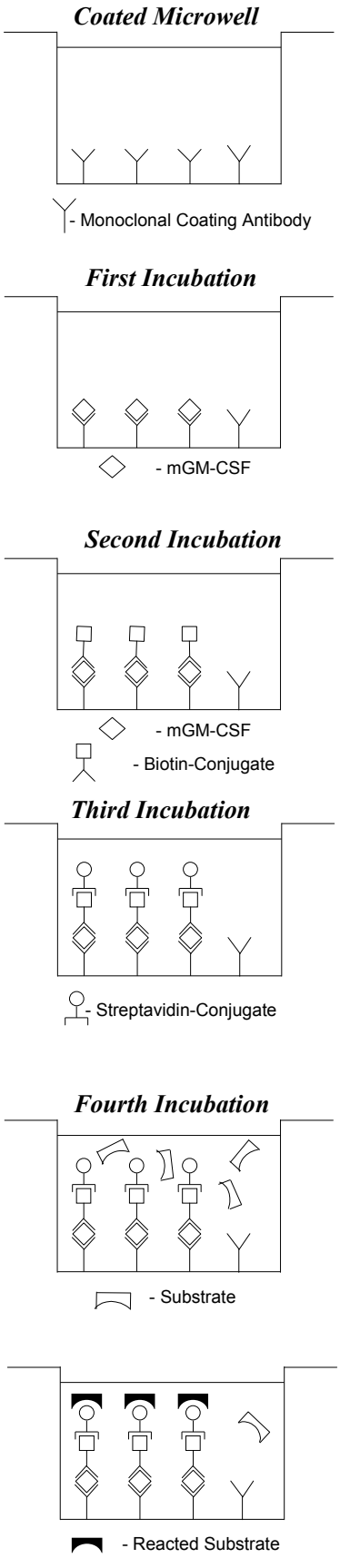
An anti-mGM-CSF monoclonal coating antibody is adsorbed onto microwells.

mGM-CSF present in the sample or standard binds to antibodies adsorbed to the microwells.

Following incubation unbound material of the standard and sample solutions is removed during a wash step. A biotin conjugated monoclonal anti-mGM-CSF antibody is added and binds to mGM-CSF captured by the first antibody.

Following incubation unbound biotin conjugated anti-mGM-CSF is removed during a wash step. Streptavidin-HRP is added and binds to the biotin conjugated anti-mGM-CSF. Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

A coloured product is formed in proportion to the amount of mGM-CSF present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven mGM-CSF standard dilutions and mGM-CSF sample concentration determined.



# 4 REAGENTS PROVIDED

1 aluminium pouch with a **Antibody Coated Microtiter Strips** with Monoclonal Antibody (rat) to murine GM-CSF

1 vial (150 µl) **Biotin-Conjugate** anti-mGM-CSF antibody[1)]

1 vial (150 µl) **Streptavidin-HRP**[1)]

2 vials **mGM-CSF Standard,** lyophilized, 500 pg/ml upon reconstitution

1 vial (5 ml) **Assay Buffer Concentrate** 20x
(PBS with 1 % Tween 20 and 10 % BSA)

1 bottle (50 ml) **Wash Buffer Concentrate** 20x
(PBS with 1% Tween 20)

1 bottle (12 ml) **Sample Diluent**

1 vial (7 ml) **Substrate Solution I** (tetramethyl-benzidine)

1 vial (7 ml) **Substrate Solution II**
(0.02 % buffered hydrogen peroxide)

1 vial (12 ml) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 ml) **Blue-Dye**

1 vial (0.4 ml) **Red-Dye**

1 vial (0.4 ml) **Green-Dye**

4 adhesive **Plate Covers**

**Reagent Labels**

**1)** It is recommended to spin vial in microcentrifuge before use to collect reagent at the bottom.

## 5 STORAGE INSTRUCTIONS

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

## 6 SPECIMEN COLLECTION

Cell culture supernatants, murine serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Clinical samples should be kept at 2° to 8°C and separated rapidly before storing at -20°C to avoid loss of bioactive mGM-CSF. If samples are to be run within 24 hours, they may be stored at 2° to 8°C. Avoid repeated freeze-thaw cycles.

For stability and suitability of samples refer to respective chapter.

# 7 MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes

- 10 µl to 1,000 µl adjustable single channel micropipettes with disposable tips

- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips

- Multichannel micropipette reservoir

- Beakers, flasks, cylinders necessary for preparation of reagents

- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)

- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)

- Glass-distilled or deionized water

- Statistical calculator with program to perform linear regression analysis

# 8 PRECAUTIONS FOR USE

- 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 statements(s) for specific advice.

- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.

- Do not mix or substitute reagents with those from other lots or other sources.

- Do not use kit reagents beyond expiration date on label.

- Do not expose kit reagents to strong light during storage or incubation.

- Do not pipette by mouth.

- Do not eat or smoke in areas where kit reagents or samples are handled.

- Avoid contact of skin or mucous membranes with kit reagents or specimens.

- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.

- Reagents containing thimerosal as preservative may be toxic if ingested.

- Avoid contact of substrate solutions with oxidizing agents and metal.

- Avoid splashing or generation of aerosols.

- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.

- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.

- Exposure to acids will inactivate the conjugate.

- Glass-distilled water or deionized water must be used for reagent preparation.

- Substrate solutions must be at room temperature prior to use.

- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents.The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.

- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

# 9 PREPARATION OF REAGENTS

## A. Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

| Number of Strips | Wash Buffer Concentrate (ml) | Distilled Water (ml) |
|---|---|---|
| 1 - 6 | 25 | 475 |
| 1 - 12 | 50 | 950 |

## B. Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer** Concentrate (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table.

| Number of Strips | Assay Buffer Concentrate (ml) | Distilled Water (ml) |
|---|---|---|
| 1 - 6 | 2.5 | 47.5 |
| 1 - 12 | 5.0 | 95.0 |

## C. Preparation of Biotin-Conjugate

Make a 1:100 dilution of the concentrated **Biotin-Conjugate** with **Assay Buffer** in a clean plastic tube as needed according to the following table:

| Number of Strips | Biotin-Conjugate (ml) | Assay Buffer (ml) |
|---|---|---|
| 1 - 6 | 0.06 | 6.0 |
| 1 - 12 | 0.12 | 12.0 |

## D. Preparation of mGM-CSF Standard

Reconstitute mGM-CSF **Standard** by addition of distilled water. Refer to the Certificate of Analysis for current volume of Distilled water needed for reconstitution of standard. Mix gently to ensure complete solubilization. Store reconstituted Standard promptly at –20°C. Discard after one week.

## E. Preparation of Streptavidin-HRP

Make a 1:100 dilution of the concentrated **Streptavidin-HRP** solution in **Assay Buffer** as needed according to the following table:

| Number of Strips | Streptavidin-HRP (ml) | Assay Buffer (ml) |
|---|---|---|
| 1 - 6 | 0.060 | 6 |
| 1 - 12 | 0.120 | 12 |

## F. TMB Substrate Solution

Using clean pipettes and containers known to be metal free, dispense an equal volume of **Substrate Solution I** into **Substrate Solution II** and swirl gently to mix. The TMB Substrate Solution may develop a yellow tinge over time. This does not seem to affect product performance. A blue colour present in the TMB Substrate Solution, however, indicates that it has been contaminated and must be discarded. The TMB Substrate Solution must be used within a few minutes after mixing. Warm to room temperature before use. Avoid direct exposure of TMB reagents to intense light and oxidizing agents during storage or incubation.

Substrate preparation according to assay size:

| Number of Strips | Substrate Solution I (ml) | Substrate Solution II (ml) |
|---|---|---|
| 1 - 6 | 3.0 | 3.0 |
| 1 - 12 | 6.0 | 6.0 |

## G. Addition of Colour-giving Dyes

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Blue-Dye, Green-Dye, Red-Dye***) can be added to the reagents according to the following guidelines:

**1. Diluent:**

Before sample dilution add the ***Blue-Dye*** at a dilution of 1:250 (see table below) to the appropriate diluent (1x) according to the test protocol. After addition of ***Blue-Dye***, proceed according to the instruction booklet.

| | |
|---|---|
| 5 ml Diluent | 20 µl ***Blue-Dye*** |
| 12 ml Diluent | 48 µl ***Blue-Dye*** |

**2. Biotin-Conjugate:**

Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of Biotin-conjugate.

| | |
|---|---|
| 6 ml Assay Buffer | 60 µl ***Green-Dye*** |
| 12 ml Assay Buffer | 120 µl ***Green-Dye*** |

**3. Streptavidin-HRP:**

Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

| | |
|---|---|
| 6 ml Assay Buffer | 24 µl ***Red-Dye*** |
| 12 ml Assay Buffer | 48 µl ***Red-Dye*** |

## 10 TEST PROTOCOL

a. Prepare reagents immediately before use and mix them thoroughly without foaming.

b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (rat) to murine mGM-CSF from holder and store in foil bag with the desiccant provided at 2°-8°C sealed tightly.

c. Wash the microwell strips twice with approximately 300 µl **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Take care not to scratch the surface of the microwells.
   After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µl of **Sample Diluent** in duplicate to all standard wells. Prepare standard dilutions by pipetting 100 µl of reconstituted (Refer to preparation of reagents) **mGM-CSF Standard**, in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µl to well B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of mGM-CSF standard dilutions ranging from 250 to 3.9 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of mGM-CSF standard dilutions:

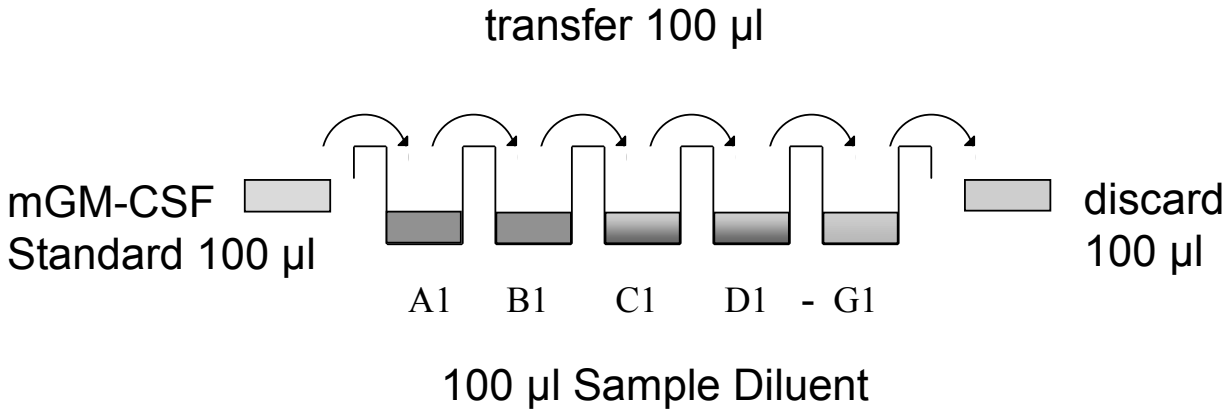



Figure 2. Diagram depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **A** | Standard 1 (250 pg/ml) | Standard 1 (250 pg/ml) | Sample 1 | Sample 1 |
| **B** | Standard 2 (125 pg/ml) | Standard 2 (125 pg/ml) | Sample 2 | Sample 2 |
| **C** | Standard 3 (62.5 pg/ml) | Standard 3 (62.5 pg/ml) | Sample 3 | Sample 3 |
| **D** | Standard 4 (31.3 pg/ml) | Standard 4 (31.3 pg/ml) | Sample 4 | Sample 4 |
| **E** | Standard 5 (15.6 pg/ml) | Standard 5 (15.6 pg/ml) | Sample 5 | Sample 5 |
| **F** | Standard 6 (7.8 pg/ml) | Standard 6 (7.8 pg/ml) | Sample 6 | Sample 6 |
| **G** | Standard 7 (3.9 pg/ml) | Standard 7 (3.9 pg/ml) | Sample 7 | Sample 7 |
| **H** | Blank | Blank | Sample 8 | Sample 8 |

e. Add 100 µl of **Sample Diluent,** in duplicate, to the blank wells.

f. Add 50 µl of **Sample Diluent** to the sample wells.

g. Add 50 µl of each **Sample**, in duplicate, to the designated wells.

h. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 2 hours on a microplate shaker set at 200 rpm.

i. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point c. of the test protocol. Proceed immediately to the next step.

j. Prepare **Biotin-Conjugate**. (Refer to preparation of reagents)

k. Add 100 µl of diluted **Biotin-Conjugate** to all wells, including the blank wells.

l. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 1 hour on a microplate shaker set at 200 rpm.

m. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point c. of the test protocol. Proceed immediately to the next step.

n. Prepare **Streptavidin-HRP**. (Refer to preparation of reagents)

o. Add 100 µl of diluted **Streptavidin-HRP** to all wells, including the blank wells.

p. Cover with a **Plate Cover** and incubate at room temperature (18° to 25°C) for 30 minutes on a microplate shaker at 200 rpm.

q. Prepare **TMB Substrate Solution** a few minutes prior to use. (Refer to preparation of reagents ).

r. Remove Plate Cover and empty wells. Wash microwell strips 4 times according to point c. of the test protocol. Proceed immediately to the next step.

s. Pipette 100 µl of mixed **TMB Substrate Solution** to all wells, including the blank wells.

t. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes, if available on a rotator set at 100 rpm. Avoid direct exposure to intense light.
**The colour development on the plate should be monitored and the substrate reaction stopped (see point u. of this protocol) before positive wells are no longer properly recordable.**
It is recommended to add the stop solution when the highest standard has developed a dark blue colour.
Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as an OD of 0.6 – 0.65 is reached.

u. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

v. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples and the mGM-CSF standards.

**Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.**

# 11 CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the mGM-CSF concentration on the abscissa. Draw a best fit curve through the points of the graph.

- To determine the concentration of circulating mGM-CSF for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding mGM-CSF concentration.

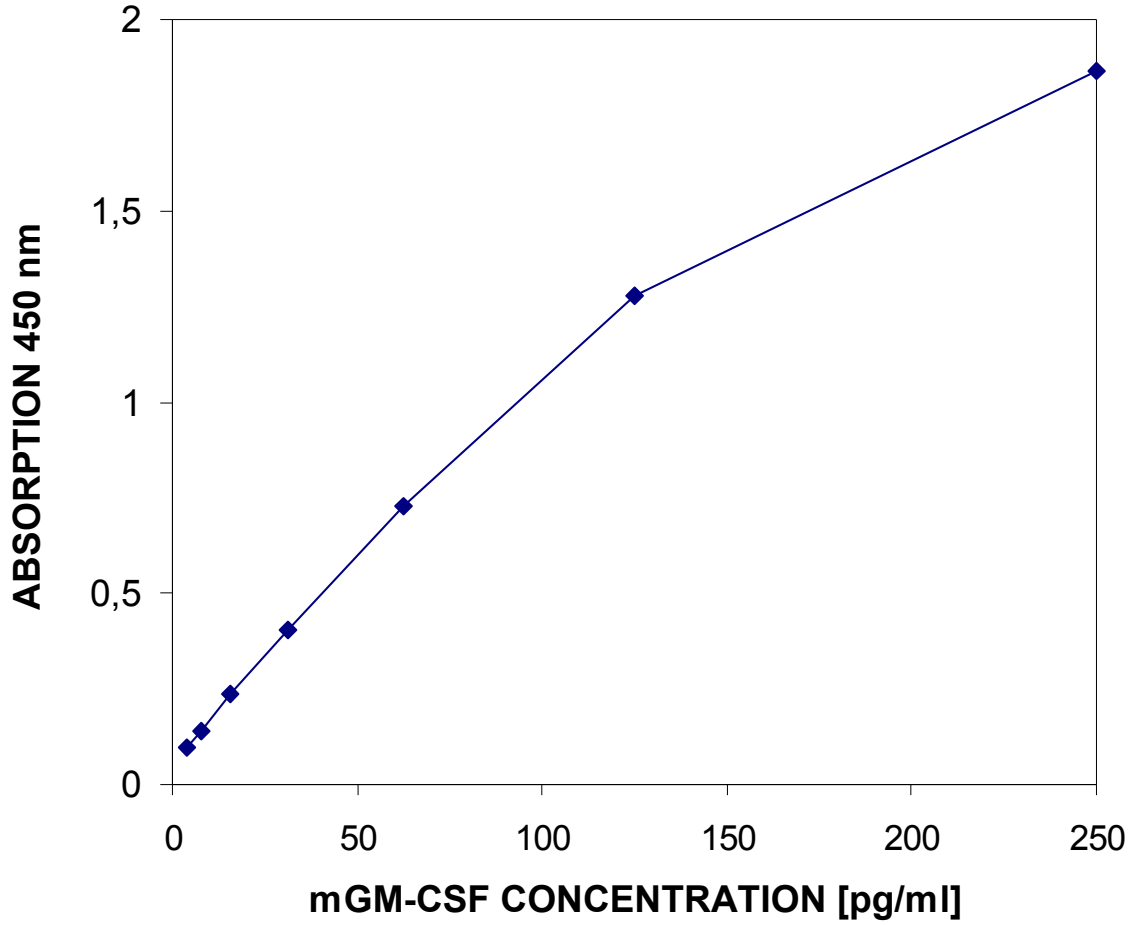
  **If samples have been diluted according to the instructions given in this manual (e.g. cell culture supernatants), the concentration read from the standard curve must be multiplied by the respective dilution factor.**

  **Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low mGM-CSF levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual mGM-CSF level.**

  It is suggested that each testing facility establishes a control sample of known mGM-CSF concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative standard curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

Figure 3. Representative standard curve for mGM-CSF ELISA. mGM-CSF was diluted in serial two-fold steps in Sample Diluent, symbols represent the mean of three parallel titrations.
Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Typical data using the mGM-CSF ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

| Standard | mGM-CSF Concentration (pg/ml) | O.D. (450 nm) | O.D. Mean | C.V. (%) |
|---|---|---|---|---|
| 1 | 250 | 1.857 | 1.866 | 0.5 |
| | 250 | 1.875 | | |
| 2 | 125 | 1.278 | 1.276 | 0.2 |
| | 125 | 1.274 | | |
| 3 | 62.5 | 0.701 | 0.726 | 3.4 |
| | 62.5 | 0.751 | | |
| 4 | 31.3 | 0.429 | 0.406 | 5.7 |
| | 31.3 | 0.383 | | |
| 5 | 15.6 | 0.237 | 0.239 | 0.6 |
| | 15.6 | 0.240 | | |
| 6 | 7.8 | 0.141 | 0.139 | 1.8 |
| | 7.8 | 0.136 | | |
| 7 | 3.9 | 0.100 | 0.098 | 2.0 |
| | 3.9 | 0.096 | | |
| Blank | | 0.039 | 0.035 | |
| | | 0.031 | | |

# 12 LIMITATIONS

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.

- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

# 13 PERFORMANCE CHARACTERISTICS

## A. Sensitivity

The limit of detection of mGM-CSF defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 1.98 pg/ml (mean of 6 independent assays).

## B. Reproducibility

### a. Intra-assay

Reproducibility within the assay was evaluated in independent experiments. The overall intra-assay coefficient of variation has been calculated to be <5%.

### b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in independent experiments by three technicians. The overall inter-assay coefficient of variation has been calculated to be <10%.

## C. Spiking Recovery

The spiking recovery was evaluated by spiking different levels of mGM-CSF into pooled normal murine serum. Recoveries were determined in two independent experiments with 4 replicates each. Observed values showed an overall mean recovery of 85%.

## D. Dilution Linearity

Murine serum spiked with different levels of mGM-CSF was assayed at four serial twofold dilutions with 4 replicates each. Experiments showed an overall mean recovery of 106 %.

## E. Sample Stability

### a. Freeze-Thaw Stability

Aliquots of spiked serum were stored frozen at –20°C and thawed up to 5 times, and mGM-CSF levels determined. There was no significant loss of GM-CSF freezing and thawing up to 5 times.

### b. Storage Stability

Aliquots of spiked serum were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the mGM-CSF level determined after 24 h. There was no significant loss of mGM-CSF immunoreactivity during storage under above conditions.

## F. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking these proteins at physiologically relevant concentrations into a mGM-CSF positive serum. There was no detectable cross reactivity.

## G. Expected Serum Values

There are no detectable mGM-CSF levels found in healthy mice. Elevated mGM-CSF levels depend on the type of immunological disorder.

# 14 REFERENCES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5. Park L. S., Martin U., Sorensen R., Luhr S., Morrissey P.J., Cosman D. and Larsen A. (1992) Cloning of the low-affinity murine granulocyte-macrophage colony-stimulating factor receptor and reconstitution of a high-affinity receptor complex. Proc. Natl. Acad. Sci. 89, 4295-4299

## 15 REAGENT PREPARATION SUMMARY

**A. Wash Buffer** Add **Wash Buffer Concentrate** 20 x (50 ml) to 950 ml distilled water

**B. Assay Buffer**

| Number of Strips | Assay Buffer Concentrate (ml) | Distilled Water (ml) |
|---|---|---|
| 1 - 6 | 2.5 | 47.5 |
| 1 - 12 | 5.0 | 95.0 |

**C. Biotin-Conjugate** Make a 1:100 dilution according to the table.

| Number of Strips | Biotin-Conjugate (ml) | Assay Buffer (ml) |
|---|---|---|
| 1 - 6 | 0.06 | 6 |
| 1 - 12 | 0.12 | 12 |

**D. Standard** Reconstitute mGM-CSF Standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial.

**E. Streptavidin-HRP**

| Number of Strips | Streptavidin-HRP (ml) | Assay Buffer (ml) |
|---|---|---|
| 1 - 6 | 0.060 | 6.0 |
| 1 - 12 | 0.120 | 12.0 |

**F. TMB Substrate Solution**

| Number of Strips | Substrate Solution I (ml) | Substrate Solution II (ml) |
|---|---|---|
| 1 - 6 | 3.0 | 3.0 |
| 1 - 12 | 6.0 | 6.0 |

# 16 TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer**
- Add 100 µl **Sample Diluent**, in duplicate, to all standard wells
- Pipette 100 µl reconstituted **mGM-CSF Standard** into the first wells and create standard dilutions ranging from 250 to 3.9 pg/ml by transferring 100 µl from well to well. Discard 100 µl from the last wells.
- Add 100 µl **Sample Diluent**, in duplicate, to the blank wells
- Add 50 µl **Sample Diluent**, in duplicate, to the sample wells
- Add 50 µl **Sample**, in duplicate, to designated wells
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C) on microplate shaker.
- Empty and wash microwell strips 4 times with Wash Buffer.
- Prepare **Biotin-Conjugate**
- Add 100 µl of diluted **Biotin-Conjugate** to all wells
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C) on microplate shaker
- Prepare **Streptavidin-HRP**
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl of diluted **Streptavidin-HRP** to all wells
- Cover microwell strips and incubate 30 minutes at room temperature (18° to 25°C) on microplate shaker
- Prepare **TMB Substrate Solution** few minutes prior to use
- Empty and wash microwell strips 4 times with **Wash Buffer**
- Add 100 µl of mixed **TMB Substrate Solution** to all wells including blank wells
- Incubate the microwell strips for 10 minutes at room temperature (18°to 25°C) on microplate shaker
- Add 100 µl **Stop Solution** to all wells including blank wells
- Blank microwell reader and measure colour intensity at 450 nm

**Note: Calculation of samples with an O.D. exceeding 2.0 may result in incorrect, low mGM-CSF levels. Such samples should be re-analyzed at higher dilution rate in order to precisely quantitate the actual mGM-CSF level.**

## NOTES

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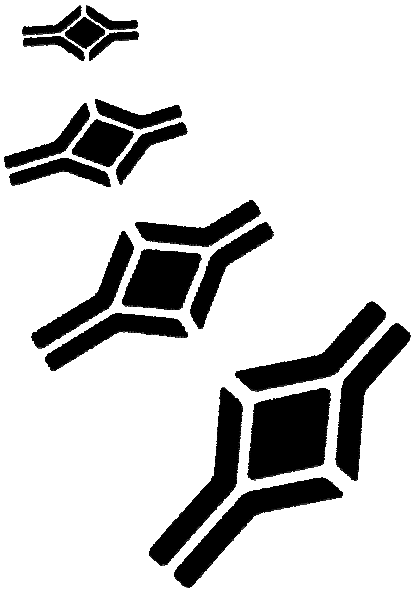


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