

# Human GM-CSF ELISA Kit

Catalog # EA100012: 1 plate

**Enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) in cell culture supernatant, serum, and plasma.**

Please read entire package insert completely before using the product.

**For research use only.**

**Not for diagnostic or therapeutic procedures.**



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## INTRODUCTION

**INTENDED USE** The OriGene human GM-CSF ELISA kit is designed to detect and quantify the level of human GM-CSF in cell culture supernatant, serum, and plasma. This kit is for research use only and not intended for diagnostic purposes.

**BACKGROUND INFORMATION** Granulocyte-macrophage colony-stimulating factor (GM-CSF), which specifically stimulates the proliferation of mouse bone marrow cells in vitro and colonies generation of granulocytes and macrophages, was first purified from mouse lung tissue-conditioned medium. Human GM-CSF is encoded by a 2.5 kb mRNA and it comprises 4 exons. Mature human GM-CSF has 127 residues and it is derived from a precursor containing a signal peptide. Human GM-CSF showed 69% nucleotide homology and 54% amino acid homology to mouse GM-CSF and it is secreted as a monomeric 23 kDa glycosylated small protein. GM-CSF is produced by various cell types including macrophages, mast cells, T cells, fibroblasts and endothelial cells.

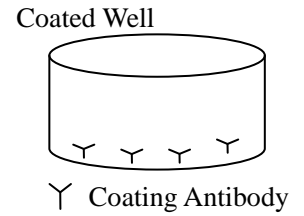
The biological activities of GM-CSF are exerted through interaction with cell surface receptors that are expressed on monocytes, macrophages, granulocytes, lymphocytes, endothelial cells and alveolar epithelial cells. The GM-CSF receptor (GM-CSFR) is composed of  $\alpha$  (CDw116; GM-CSFR $\alpha$ ) and  $\beta$  (GM-CSFR $\beta$ c) chains. The  $\beta$  chain is common to receptors for GM-CSF, IL-3, and IL-5.

GM-CSF is an important hematopoietic growth factor and immune modulator. It stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes. GM-CSF also has profound effects on the functional activities of various circulating leukocytes.

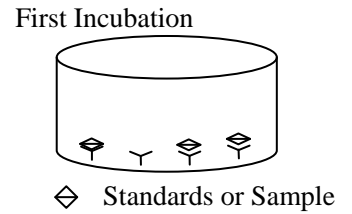
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**PRINCIPLE OF THE ASSAY**

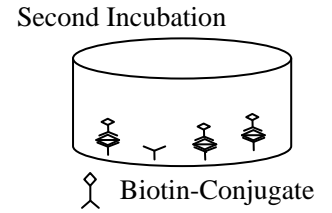
A monoclonal antibody specific for human GM-CSF has been coated onto the wells of the microtiter strips provided.



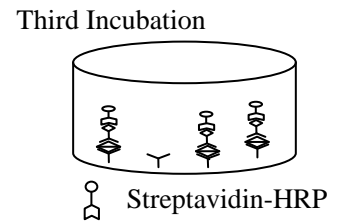
Standards and samples are pipetted into the wells and human GM-CSF binds to the immobilized antibody.



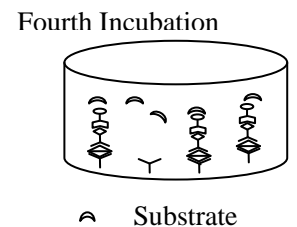
After incubation, unbound substances is removed during a wash step, a biotin-conjugated anti-human GM-CSF antibody is added and binds to human GM-CSF captured by the first antibody.



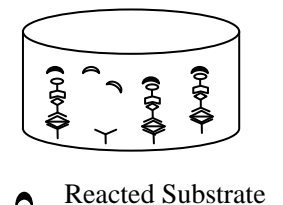
After incubation, unbound biotin-conjugated anti-human GM-CSF antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human GM-CSF antibody.



After incubation, unbound streptavidin-HRP is removed during a wash step, and a chromogenic substrate solution is added to the wells and color develops in proportion to the amount of GM-CSF bound in the initial step.



A colored product is formed and the reaction is terminated by the addition of stop solution. The intensity of the color is measured spectrophotometrically at 450 nm.



## MATERIALS AND METHODS

### REAGENTS PROVIDED

Description		Part#
<b>Human GM-CSF Ab Coated Wells</b> , 96-well polystyrene microplate (12 strips of 8 wells)	1 plate	03921
<b>Human GM-CSF Standard</b> , lyophilized recombinant Human GM-CSF Refer to vial label for quantity and reconstitution volume	2 bottles	03931
<b>Assay Solution</b> , 25 mL per bottle with preservatives	1 bottle	AS04
<b>Human GM-CSF Biotin Conjugate</b> (biotin-labeled anti-human GM-CSF antibody), 11 mL per bottle with preservatives	1 bottle	03941
<b>Streptavidin-horseradish peroxidase (HRP) Concentrate (100X)</b> , 150 µL per vial with preservatives	1 vial	03951
<b>Streptavidin-HRP Diluent</b> , 12 mL per bottle with preservatives	1 bottle	HD01
<b>Wash Solution Concentrate (10X)</b> , 75 mL with preservatives	2 bottles	WS01
<b>Chromogen Solution (Tetramethylbenzidine, TMB)</b> , 12 mL per bottle	1 bottle	CT01
<b>Stop Solution</b> , 12 mL per bottle	1 bottle	SS01
<b>Plate Covers</b> , Adhesive strips	4 strips	

Store all reagents at 2 to 8°C.

**OTHER  
SUPPLIES  
REQUIRED**

1. A standard ELISA plate reader for absorbance at 450 nm.
2. Calibrated adjustable precision pipettes (single channel and multi channel), preferably with disposable plastic tips.
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software or graph paper.
6. Polypropylene tubes.
7. Graduated cylinders and calibrated beakers in various sizes.

**SAFETY**

All biological materials and blood components should be handled as potentially hazardous. Follow universal precautions when handling and disposing of infectious agents.

**PRECAUTIONS  
FOR USE**

1. This kit is for research use only, not for diagnostic or therapeutic procedures.
2. All samples and reagents must be at room temperature (20 to 25°C) before use. The kit components should be refrigerated after use.
3. The kit should not be used beyond the expiration date.
4. Human GM-CSF Ab coated wells should be allowed to come to room temperature before opening the foil bag. After the desired number of strips has been removed, immediately reseal the remaining strips and store at 2 to 8°C.
5. Samples should be thawed completely and mixed well prior to analysis. Avoid multiple freeze-thaw cycles of frozen samples.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are > 500 pg/mL of human GM-CSF must be diluted with **Assay Solution** and reanalyzed.
8. Maintain a consistent order of addition from well-to-well when pipetting reagents. This ensures equal incubation times for all wells.
9. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
10. Do not mix reagents from different kit lots.
11. Read absorbance within 30 minutes of assay completion.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
13. Take care not to contaminate the **Chromogen Solution**. If the solution is blue before use, DO NOT USE.

**SAMPLE  
PREPARATION**

**Cell Culture Supernatant** Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Serum** If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 10 minutes at  $1000\times g$ . Remove serum and assay promptly or aliquot and store the samples at  $\leq -20^{\circ}\text{C}$ . Avoid multiple freeze-thaw cycles. If serum separator tubes are not being used, allow samples to clot overnight at  $2-8^{\circ}\text{C}$ . Centrifuge for 10 minutes at  $1000\times g$ . Remove serum and assay promptly or aliquot and store the samples at  $\leq -20^{\circ}\text{C}$ . Avoid multiple freeze-thaw cycles.

**Note:** It is recommended that all samples be diluted in **Assay Solution**, and the exact dilution must be determined empirically.

**REAGENT  
PREPARATION**

**A. Human GM-CSF Standard**

1. Reconstitute standard to 1000 pg/mL with **distilled water** (see reconstitution instructions on label). Mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Prepare standards just before use and use within one hour of reconstitution. Do not store reconstituted standards.
2. Add 0.3 mL of **Assay Solution** to each of 7 tubes labeled 500 , 250, 125, 62.5, 31.2, 15.6 and 7.8 pg/mL of Human GM-CSF.
3. Make serial dilutions of the standard as described in the following table. Mix thoroughly between steps.

**B. Wash Solution**

Allow **Wash Solution Concentrate (10X)** to warm up to room temperature and mix gently. Then make a 1:10 dilution of **Wash Solution Concentrate (10X)** with deionized or distilled water in a clean plastic tube as needed. Label as **Wash Solution**. Store both the concentrate and the **Wash Solution** in the refrigerator. The diluted buffer should be used within two weeks.

### Dilution of Human GM-CSF Standard

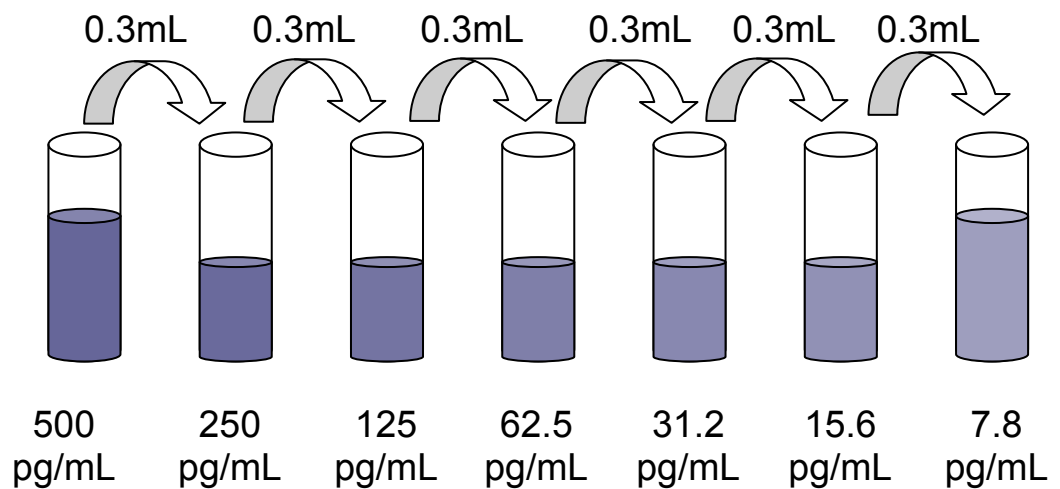
Standard	Add	Into
1000 pg/mL	Prepare as described in Step 1.	
500 pg/mL	0.3 mL of the 1000 pg/mL std.	0.3 mL of Assay Solution
250 pg/mL	0.3 mL of the 500 pg/mL std.	0.3 mL of Assay Solution
125 pg/mL	0.3 mL of the 250 pg/mL std.	0.3 mL of Assay Solution
62.5 pg/mL	0.3 mL of the 125 pg/mL std.	0.3 mL of Assay Solution
31.2 pg/mL	0.3 mL of the 62.5 pg/mL std.	0.3 mL of Assay Solution
15.6 pg/mL	0.3 mL of the 31.2 pg/mL std.	0.3 mL of Assay Solution
7.8 pg/mL	0.3 mL of the 15.6 pg/mL std.	0.3 mL of Assay Solution
0 pg/mL		0.3 mL of Assay Solution

**REAGENT  
PREPARATION**

**C. Streptavidin-HRP Solution**

Make a 1:100 dilution of the **Streptavidin-HRP Concentrate (100X)** with **Streptavidin-HRP Diluent** in a clean plastic tube as needed. Label as **Streptavidin-HRP Solution**. **Streptavidin-HRP Solution** should be used within 30 minutes after dilution.

# of 8-well strips	Volume of Streptavidin-HRP concentrate (100X)	Volume of Streptavidin-HRP Diluent
2	20 $\mu$ L	2 mL
4	40 $\mu$ L	4 mL
6	60 $\mu$ L	6 mL
8	80 $\mu$ L	8 mL
10	100 $\mu$ L	10 mL
12	120 $\mu$ L	12 mL



**ASSAY  
PROCEDURE**

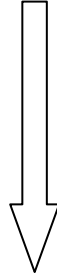
Allow all reagents and samples to warm up to room temperature before use. It is recommended that all standards and samples be assayed in duplicate.

1. Determine the number of strips needed for the assay and remove excess microplate strips from the plate frame, return them to the foil pouch, and reseal.
2. Prepare standards and samples with appropriate diluents. (Prepare **Human GM-CSF Standard** according to **REAGENT PREPARATION A.**)
3. Add 100  $\mu\text{L}$  of reconstituted standards or dilutions of samples in duplicate to each well. Add 100  $\mu\text{L}$  of **Assay Solution** in duplicate to the blank wells. Seal the plate with **Plate Covers** and incubate at room temperature for 2 hours.
4. Aspirate each well and wash 4 times. Wash by filling each well with **Wash Solution** (300  $\mu\text{L}$ ) using a multi-channel pipette, squirt bottle, manifold dispenser or autowasher. Allow the **Wash Solution** to soak for about 10-20 seconds before aspiration. After the last wash step, the plate is inverted and tapped dry on absorbent pad or paper towel. A thorough washing of the plate is extremely important to reduce background. (Prepare **Wash Solution** according to **REAGENT PREPARATION B.**)
5. Add 100  $\mu\text{L}$  of **Human GM-CSF Biotin Conjugate** to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
6. Aspirate each well and wash according to Step 4.
7. Add 100  $\mu\text{L}$  of **Streptavidin-HRP Solution** to each well. Cover with a new adhesive strip. Incubate for 30 minutes at room temperature. (Prepare **Streptavidin-HRP Solution** according to **REAGENT PREPARATION C.**)
8. Aspirate each well and wash according to Step 4.
9. Add 100  $\mu\text{L}$  of **Chromogen Solution** (the substrate) to each well. Incubate for 30 minutes at room temperature and **in the dark**.
10. Add 100  $\mu\text{L}$  of **Stop Solution** to each well. The color in the well should change from blue to yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read the absorbance of each well at 450 nm within 30 minutes after adding the **Stop Solution**.

**ASSAY  
PROCEDURE  
SUMMARY**

**Add 100  $\mu$ L of reconstituted standards or samples to each well**

Incubate for 2 hours at RT  
Aspirate and wash 4 times



**Add 100  $\mu$ L of Human GM-CSF Biotin Conjugate to each well**

Incubate for 1 hour at RT  
Aspirate and wash 4 times



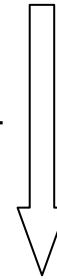
**Add 100  $\mu$ L of Streptavidin-HRP Solution to each well**

Incubate for 30 minutes at RT  
Aspirate and wash 4 times



**Add 100  $\mu$ L of Chromogen Solution to each well**

Incubate for 30 minutes at RT



**Add 100  $\mu$ L of Stop Solution to each well and read at 450 nm**

**CALCULATION OF RESULTS**

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

**Manual Plotting:**

Plot on graph paper the absorbance of the standards against the standard concentration. Known concentrations of human GM-CSF are plotted on the X-axis and the corresponding absorbances on the Y-axis. The standard curve should result in a straight line that shows a direct relationship between human GM-CSF concentrations and the corresponding absorbances. The concentration of human GM-CSF in samples may be determined by plotting the sample absorbances on the Y-axis, then drawing a horizontal line to intersect with the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human GM-CSF concentration.

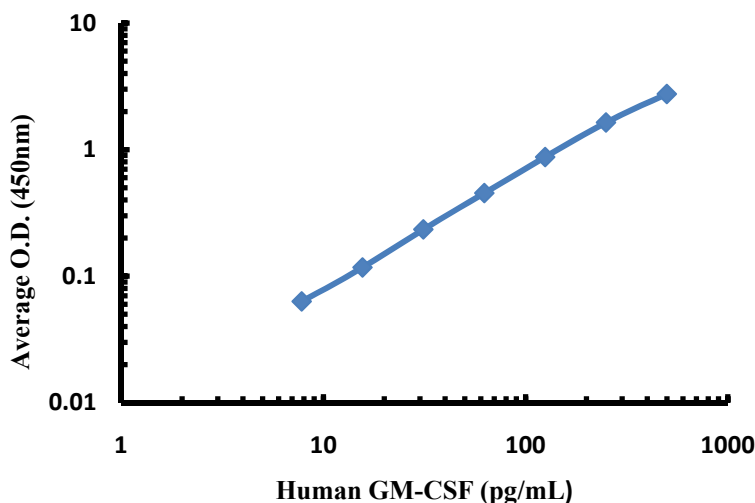
Note: Samples producing signals greater than that of the highest standard (500 pg/mL) should be diluted in **Assay Solution** and reanalyzed. Multiply the measured concentration by the appropriate dilution factor.

**Plate Reader:**

An alternative approach is to use an ELISA curve fitting software. A linear curve plot is expected to produce the best fit of the resulting sample concentrations.

**TYPICAL DATA**

This standard curve is provided for demonstration only. A standard curve should be generated for each assay plate.



Standard Human GM-CSF (pg/mL)	Average O.D. (450 nm)	Corrected
0	0.125	---
7.8	0.188	0.063
15.6	0.242	0.117
31.2	0.359	0.234
62.5	0.577	0.452
125	0.998	0.873
250	1.763	1.638
500	2.877	2.752

## PERFORMANCE OF CHARACTERISTICS

### SPECIFICITY

This assay recognizes both recombinant and natural human GM-CSF. Human peripheral blood mononuclear cells ( $2 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured and stimulated with 50 ng/mL PMA and 1 µg/mL Ionomycin. Aliquots of the culture supernatant were removed and assayed for levels of natural GM-CSF. Human GM-CSF is up-regulated in the supernatant of stimulated cells. Buffered solutions of a panel of cytokines were assayed at 50ng/mL with the OriGene human GM-CSF kit. No significant cross-reactivity or interference was observed with the following: human IL-4, IL-6, IL-12p40, IL-12p70, IL-17A, IL-13, IL-21, IFN- $\gamma$ , HGF, VEGF, MCP-1; mouse GM-CSF, IL-2, IL-4, IL-5, IL-10, IL-12p70, IFN- $\gamma$ , VEGF, TNF- $\alpha$ .

Conditions	Day 1
unstimulated	ND
PMA(50 ng/mL) + Ionomycin(1 µg/mL)	1543.9 pg/mL

ND=No Detectable

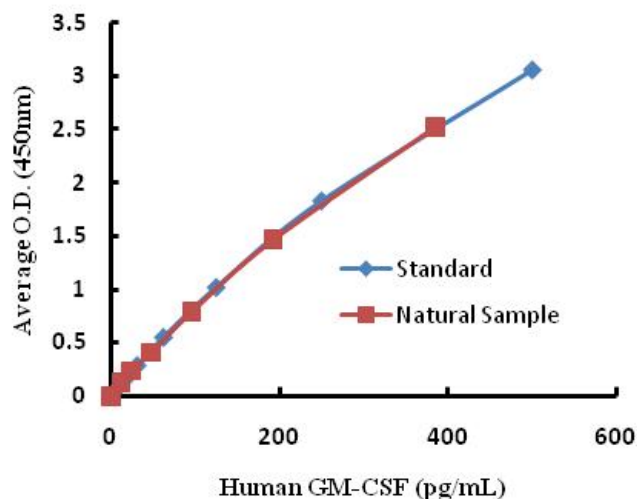
### SENSITIVITY

The minimum detectable dose of human GM-CSF is <2 pg/mL. This was determined by adding three standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

### RECOVERY

The recovery of natural GM-CSF from human cell culture supernatant added to human serum averaged 94%, ranging from 88-99%.

### PARALLELISM



The human GM-CSF standard and natural sample from human cell culture supernatant were tested in dilutions and compared. Parallelism between standard and natural human GM-CSF was demonstrated in this comparison.

**LINEARITY  
OF DILUTION**

To assess the linearity of the assay, samples with 1543.9 pg/mL of measured human GM-CSF were initially diluted 1:2, then serially diluted in **Assay Solution** such that measured values fell within the dynamic range of the assay. Results demonstrated linearity of dilution within the assay range.

<b>Dilutions</b>	<b>Cell Culture Supernatant</b>		
	<b>Measured ( pg/mL )</b>	<b>Expected ( pg/mL )</b>	<b>% Expected</b>
1/4	386.0	386.0	100.0%
1/8	191.4	193.0	99.2%
1/16	92.5	96.5	95.8%
1/32	44.7	48.2	92.7%

**PRECISION****Intra-assay Precision:**

Three samples of known concentration were tested in 84 wells (28 wells for each sample) on one plate to assess intra-assay precision.

<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>
Mean (pg/mL)	100.4	274.9	465.4
SD	4.0	8.1	13.4
CV (%)	4.0	3.0	2.9

**Inter-assay Precision:**

Three samples of known concentration were tested in 84 wells from separate plates to assess inter-assay precision.

<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>
Mean (pg/mL)	102.5	275.1	467.1
SD	6.1	16.5	20.6
CV (%)	6.0	6.0	4.4

SD = Standard Deviation

CV = Coefficient of Variation

## REFERENCES

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4. Griffin JD., et al. (1990) Int J Cell Cloning 1990; 8 Suppl 1:35-44; discussion 44-5.
5. Cousins DJ., et al. (1994) Am J Respir Crit Care Med 150:S50-53.
6. Nimer SD., et al. (1995) Stem Cells 13:324-335.
7. Griffin JD., et al. (1990) J Immunol 145:576-584.
8. Miyajima A., et al. (1992) 10:126-134.

# Notes of Experimental Design

A grid of 96 circles arranged in 8 columns and 12 rows. The columns are labeled A through H at the bottom, and the rows are labeled 1 through 12 on the left side. The grid is enclosed in a rectangular border with a small notch at the bottom-left corner.

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								