Human GM-CSF ELISA Kit

For the quantitative determination of human granulocyte macrophage colony stimulating factor (GM-CSF) concentrations in serum, plasma, cell culture supernatant, and other biological fluids.

Catalogue Number: EL10020

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE

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S7.5 (04) GM-CSF

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INTENDED USE

This Human GM-CSF ELISA Kit is to be used for the *in vitro* quantitative determination of human granulocyte macrophage colony stimulating factor (GM-CSF) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended for LABORATORY RESEARCH ONLY and is not for use in diagnostic or therapeutic procedures.

INRODUCTION

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) is a member of the hematopoietic cytokine family, which includes interleukin-3 (IL-3) and interleukin-5 (IL-5). It is a pleiotropic cytokine that was one of the first growth factors characterized and shown to be necessary for the proliferation, differentiation, activation, and survival of hematopoietic cells. Human GM-CSF precursor (144 a.a.) is cleaved at the amino-terminal end to form a mature polypeptide (23 kDa, 127 a.a) that contains two intramolecular disulfide bonds, which are important for biological activity and two potential N-glycosylation sites. A single gene on chromosome 5 codes for the human GM-CSF protein. Human GM-CSF shows 56-60% amino acid (a.a) homology to murine GM-CSF but does not exhibit cross-species biological activity or receptor binding.[1,2,3] Glycosylation does not appear to be essential for biological activity, since recombinant GM-CSF unlike native GM-CSF is non-glycosylated and it still retains high biologic activity. However, this glycoprotein does show a decrease in affinity for its receptor as a result of non-glycosylation.[2]

Human GM-CSF is different from other family members in that it can be produced and acts upon a much wider range of cell types. T-lymphocytes, B-lymphocytes, monocytes/macrophages, endothelial cells, fibroblasts, stromal cells, mesothelial cells, keratinocytes, osteoblasts, uterine epithelial cells, synoviocytes, mast cells, and various solid tumours produce GM-CSF. Usually a cytokine, inflammatory agent, or antigen is needed to stimulate the above cells to synthesize GM-CSF.[2,3] For human GM-CSF to exert its biologic effects it will bind to a single class of cell surface receptors on hematopoietic and non-hematopoietic cells.[4] The GM-CSF receptor has been cloned[3] and, the α and β chains (80 kDa and 130 kDa) were found to members of the hematopoietin receptor family.

Numerous studies have shown diverse *in vitro* biological effects of GM-CSF on various cell types. GM-CSF can bind to pluripotent hematopoietic stem cells causing the proliferation and differentiation of various progenitor cells such as granulocyte and macrophage[3], whereas eosinophil, erythroid and megakaryocyte colony formation is stimulated at much higher concentrations.[2,3,5] GM-CSF is also required for growth and differentiation of typical dendritic cells from human bone marrow[2,6], causes activation and prolonged survival of mature hematopoietic cells[2,3], and activates mature neutrophils and eosinophils causing antibody dependent cellular cytotoxicity, phagocytosis, superoxide generation. Also, GM-CSF stimulates macrophage production of TNF, M-CSF, G-CSF, and IL-1, intensifies killing by granulocytes and macrophages[3], and increases HIV-1

replication at the post-transcriptional level.[7] GM-CSF binds to non-hematopoietic cells causing the proliferation and/or migration of fibroblast, endothelial, and various tumour cell lines.[8,9] The significance of GM-CSF receptor expression on these non-hematopoietic cell types is unknown. Very little is known about the *in vivo* biological effects of GM-CSF in various pathological states. However *in vivo* studies showed a significant eosinophilic response and macrophage granuloma formation accompanied with tissue damage when GM-CSF was overexpressed in the rat lung**.** Thus role GM-CSF may play a role in the development of fibrotic reactions.[10] *In vivo*, GM-CSF induces the upregulation of CD11b on neutrophils, induces temporary neutrophil sequestration in the lung, followed by specific granule release, and enhanced *ex vivo* production of superoxide anion on neutrophils.[11]

Various pathological conditions are associated with increased GM-CSF levels. These include: lung cancer,[12] acute mylogenous leukemia,[13] tumour related thrombocytosis,[14] myelodysplastic syndrome (MDS),[15] thrombocytopenia,[16]and psoriasis.[17] GM-CSF expression is increased in bronchial asthma and lung inflammatory diseases;[9,18] non-allergic respiratory diseases such as eosinophil pneumonia, hypersensitivity pneumonitis, iodiopathic pulmonary fibrosis, sarcoidosis, cryptogenic organizing pneumonia, HIV infection,[9] rheumatoid arthritis, and systemic lupus erythmatosus.[19] GM-CSF shows therapeutic value by accelerating neutrophil recovery in disease induced myelosuppression such as bone marrow transplantation, chemotherapy, and infectious disease.[2,3] It is suggested that a GM-CSF may be useful in autologous bone marrow transplantation to detect GM-CSF toxicity for the diagnosis of post-transplant liver disease[20] and in gestational trophoblastic disease (GTD) for the early identification of high risk choriocarcinoma cases.[21]

This GM-CSF ELISA is a 2.5-hour solid phase immunoassay readily applicable to measure GM-CSF in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 500 pg/mL. It showed no cross reactivity with other cytokines tested. This GM-CSF ELISA is expected to be effectively used for further investigations into the relationship between GM-CSF and the various conditions mentioned above.

PRINCIPLE OF THE ASSAY

This GM-CSF enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for GM-CSF. Standards or samples are then added to the appropriate microtiter plate wells and incubated. GM-CSF, if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound GM-CSF and other components of the sample. In order to quantitatively determine the amount of GM-CSF present in the sample, a standardized preparation of horseradish peroxidase HRP-conjugated monoclonal antibody specific for GM-CSF is added to each well to "sandwich" the GM-CSF immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain GM-CSF and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 $\pm$ 2 nm.

In order to measure the concentration of GM-CSF in the samples, this kit includes two diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing). According to the testing system, the provided standard is diluted (2-fold dilution series) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D.) versus GM-CSF concentration (pg/mL). The concentration of GM-CSF in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED
All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **GM-CSF MICROTITER PLATE** (Part EL20-1) ***96 wells***
   Pre-coated with anti-human GM-CSF monoclonal antibody.

2. **GM-CSF CONJUGATE** (Part EL20-2) ***12 mL***
   Anti-human GM-CSF monoclonal antibody conjugated to horseradish peroxidase with preservative.

3. **GM-CSF STANDARD** (Part EL20-3) ***2 vials***
   Recombinant human GM-CSF (1ng/vial) in a buffered protein base with preservative, lyophilized.

4. **CALIBRATOR DILUENT I** (Part EL20-4) ***25 mL***
   Animal serum with buffer and preservative. *For serum/plasma testing*.

5. **CALIBRATOR DILUENT II** (Part EL20-5) ***25 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

6. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

7. **SUBSTRATE A** (Part EL20-6) ***10 mL***
   Buffered solution with H_2O_2

8. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

9. **STOP SOLUTION** (Part 30008) ***14 mL***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 µL and 50-200 µL for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm±2 nm)
8. Automatic microtiter plate washer or squirt bottle
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave 60 min. at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING, AND STORAGE

a). **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.

b). **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4° C) and serum extracted.

c). **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4° C) to remove any particulates.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, cell culture, and cell culture supernatant samples to be used within 24-48 hour may be stored at 2-8 C. Otherwise, samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable 1 month at 2-8°C. Mix well before use.

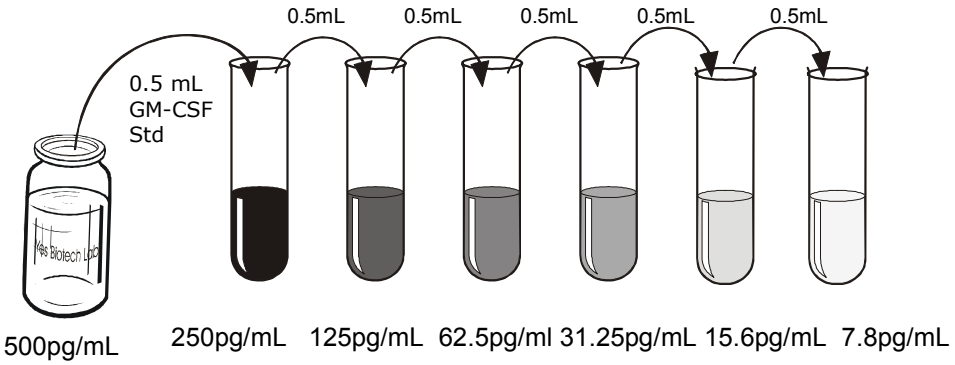
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	3.0	3.0	6.0
4 strips (32 wells)	6.0	6.0	12.0
6 strips (48 wells)	8.0	8.0	16.0
8 strips (64 wells)	10.0	10.0	20.0
10 strips (80 wells)	12.0	12.0	24.0
12 strips (96 wells)	14.0	14.0	28.0

3. **GM-CSF Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute GM-CSF Standard with 2.0 mL of Calibrator Diluent. This reconstitution produces a stock solution of 500 pg/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The GM-CSF standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles; aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (7.8 to 500pg/mL) as illustrated below. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The diluted GM-CSF Standard will serve as the high standard (500 pg/mL) and the Calibrator Diluent will serve as the zero standard (0 pg/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer and GM-CSF Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided by used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents		**Wells**	Contents	
1A, 1B	Standard 1 - **0 pg/mL**	(S1)	**2C, 2D**	Standard 6 - **125 pg/mL**	(S6)
1C, 1D	Standard 2 - **7.8 pg/mL**	(S2)	**2E, 2F**	Standard 7 - **250 pg/mL**	(S7)
1E, 1F	Standard 3 - **15.6 pg/mL**	(S3)	**2G, 2H**	Standard 8 - **500 pg/mL**	(S8)
1G, 1H	Standard 4 - **31.25 pg/mL**	(S4)	**3A-12H**	GM-CSF samples	
2A, 2B	Standard 5 - **62.5 pg/mL**	(S5)			

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	1	5	9	13	17	21	25	29	33	37
B	S1	S5	1	5	9	13	17	21	25	29	33	37
C	S2	S6	2	6	10	14	18	22	26	30	34	38
D	S2	S6	2	6	10	14	18	22	26	30	34	38
E	S3	S7	3	7	11	15	19	23	27	31	35	39
F	S3	S7	3	7	11	15	19	23	27	31	35	39
G	S4	S8	4	8	12	16	20	24	28	32	36	40
H	S4	S8	4	8	12	16	20	24	28	32	36	40

2. Add 100µL of Standard or Sample to the appropriate well of the antibody pre-coated wells of the Microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

4. Add 100µL Conjugate to each well. Cover and incubate for 1 hour at room temperature.

5. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

6. Repeat wash procedure as described in Step 3.

7. Add 100µL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100µL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of GM-CSF in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding GM-CSF concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values are subtracted by the mean value of the zero-standard (0 pg/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of GM-CSF in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding GM-CSF concentration. If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

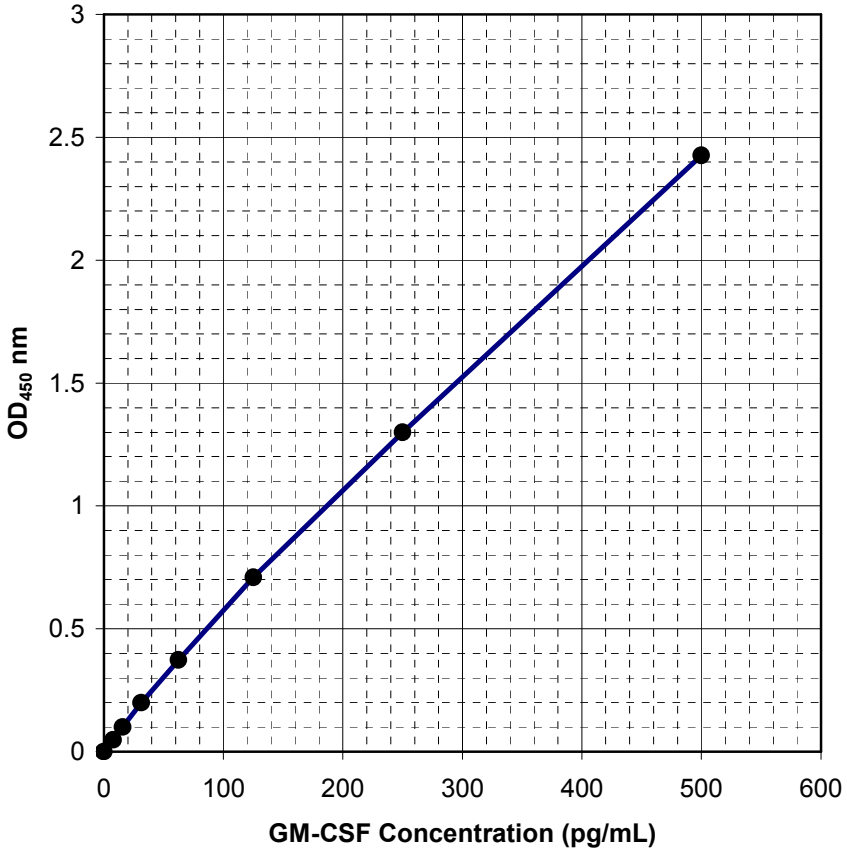
TYPICAL DATA

Results of a typical standard run of a GM-CSF ELISA are shown. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain own standard curve.

EXAMPLE

The following data was obtained for a standard curve using Calibrator Diluent I.

Standard (pg/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.014, 0.015	0.015	0
7.8	0.061, 0.065	0.063	0.048
15.6	0.114, 0.115	0.115	0.100
31.25	0.211, 0.217	0.214	0.199
62.5	0.387, 0.389	0.388	0.373
125	0.720, 0.780	0.725	0.710
250	1.312, 1.318	1.315	1.300
500	2.437, 2.447	2.442	2.427



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To determine within-run precision, three different samples of known concentration were assayed by replicates of twenty in 1 assay.

	Calibrator Diluent I Assay		
Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	16.25	63.7	226.6
Standard Deviation (pg/mL)	0.7	3.02	12.6
Coefficient of Variation (%)	*4.3*	*4.7*	*5.5*

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of know concentration were assayed by replicates on 20 different assays.

	Calibrator Diluent I Assay		
Sample	**1**	**2**	**3**
n	20	20	20
Mean (pg/mL)	8.85	55.7	243.5
Standard Deviation (pg/mL)	0.88	3.58	20.4
Coefficient of Variation (%)	*9.9*	*6.4*	*8.3*

3. **RECOVERY**
   The recovery of GM-CSF spiked to seven different levels in five test samples throughout the range of the assay was evaluated. All samples were mixed and assayed in duplicate.

Sample Type	***Average Recovery (%)***	***Range (%)***
Cell Culture Media	108.0	92.0-128.0
Serum	80.4	72.8-100.0
EDTA plasma	100.3	86.8-128.0
Heparin plasma	93.3	80.0-125.6
Citrate plasma	101.9	86.0-128.0

4. **SENSITIVITY**
   The minimum detectable dose of GM-CSF using a standard curve generated with Calibrator Diluent I is *1.5-2.0 pg/mL* and using Calibrator Diluent II is *2.0 pg/mL*.

5. **SPECIFICITY**
   This sandwich ELISA recognizes both natural and recombinant human GM-CSF. This kit exhibits no significant cross-reactivity with human; TGF, MCP-1, MCP-3, M-CSF, EGF, IL-1β, IL-8, IL-16, and TNF-α.

6. **CALIBRATION**
   This immunoassay is calibrated against NIBCS/WHO First Standard code No. 88/646

7. **EXAMPLE FOR TESTING OF CELL CULTURE SUPERNATANTS**

Activation Condition	***GM-CSF Concentration (pg/mL)***
Monocyte supernate, 1% BSA	2.0
Monocyte supernate, 1% BSA, 100ng/mL LPS	210.0

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