

# Human GM-CSF ELISpot Kit

For the quantitation of single cells releasing human GM-CSF.

Catalogue Number: SL10020E

*96 tests*

FOR LABORATORY RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.



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## INTENDED USE

Human GM-CSF enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human GM-CSF.

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## INTRODUCTION

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.<sup>1,2,3</sup> 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.<sup>2</sup>

Human GM-CSF is different from other family members in that it can be produced and act 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.<sup>2,3</sup> 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.<sup>4</sup> The GM-CSF receptor has been cloned<sup>3</sup> and, the  $\alpha$  and  $\beta$  chains (80 kDa and 130 kDa) were found to members of the hematopoietin receptor family.

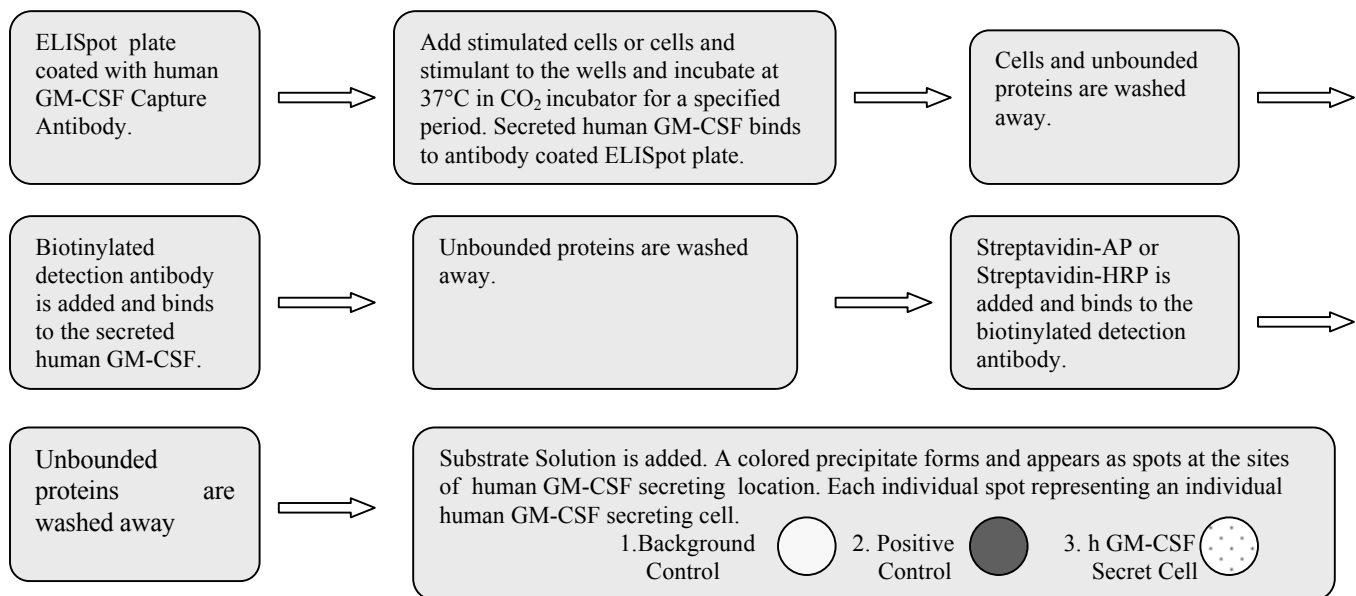
Numerous studies have shown diverse *in vitro* biological effects of GM-CSF on various cell types. GM-CSF can bind to pluripotent hematopoietic stem cells causing the proliferation and differentiation of various progenitor cells such as granulocyte and macrophage<sup>3</sup>, whereas eosinophil, erythroid and megakaryocyte colony formation is stimulated at much higher concentrations.<sup>2,3,5</sup> GM-CSF is also required for growth and differentiation of typical dendritic cells from human bone marrow<sup>2,6</sup>, causes activation and prolonged survival of mature hematopoietic cells<sup>2,3</sup>, 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<sup>3</sup>, and increases HIV-1 replication at the post-transcriptional level.<sup>7</sup> GM-CSF binds to non-hematopoietic cells causing the proliferation and/or migration of fibroblast, endothelial, and various tumour cell lines.<sup>8,9</sup> 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.<sup>10</sup> *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.<sup>11</sup>

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

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human GM-CSF.

### PRINCIPLES OF THE ASSAY



## REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

Name (Part No.)	Size	Description	Usage and Storage
1) ELISpot Plates (1X 96tests, Part SL10020E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human GM-CSF monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10020E-2)	1 Vial	Lyophilized recombinant human GM-CSF (2ng/vial)	Reconstitute 1 vial in 250 µL Cell Culture Media before use. Use in 1 hour. The final concentration is 8 ng/mL.
3) 20 X Wash Buffer Concentrated (Part SL10020E-3)	1 X 60mL	—	Add 1 volume of 20X Wash Buffer Concentrated to 19 volume of deionized water/distilled water. Use in 1 week. Stored at room temperature.
4) Human GM-CSF Detection Antibody (Part SL 10020E-4)	1 x 11mL	Biotinylated mouse anti-human GM-CSF monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part SL 10020E-5)	1 Vial	120µL 100 x Concentrated Alkaline Phosphatase labeled Streptavidin.	Add 1 volume of Concentrated Streptavidin - AP to 100 volumes of Streptavidin – AP Diluent (Part SL 10020E-6) before use. Use in 1 month. Stored at 2-8 °C.
6) Streptavidin – AP Diluent (Part SL 10020E-6)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
7) Substrate Solution (Part SL 10020E-7)	1 x 11mL	BCIP/NBT Substrate Solution.	Ready to use.

## MATERIALS REQUIRED BUT NOT SUPPLIED

1. Pipettes with disposable tips, bottles, test tubes and racks, graduated cylinders, absorbent paper, and squirt bottle.
2. 37°C CO<sub>2</sub> incubator.
3. Deionized or distilled water.
4. Dissection microscope or ELISpot reader.

## PRECAUTIONS

1. Allow kit reagents and materials to reach room temperature (20-25°C) before use.
2. Do not use kit components beyond their expiration date. Do not substitute reagents from one kit lot to another.
3. The toxicity of the Substrate Solution is not currently known, wear gloves to avoid contact with skin. Follow local, state and federal regulations to dispose of used Substrate Solution.

4. If 20 x Wash Buffer Concentrated is stored at lower temperature (2-8 °C), crystals may form which must be dissolved by warming prior to use.
5. When samples are added to the wells, don't let the pipette tips contact the membrane.
6. Don't let the plate dry during the assay.
7. In order to avoid edge effect don't stack plates during cell incubation.
8. Avoid move the plate during cells incubation period.
9. Don't dry the plate at a temperature higher than 37° C.
10. Spots can't be counted accurately until PVDF membranes were completely dry.

## **SAMPLE PREPARATION**

Each researcher should optimize cell separation method, stimulant, stimulation mode and incubation time.

A recommended method to stimulate human GM-CSF secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add 10<sup>5</sup> /mL PBMCs in 1ug / mL lipopolysaccharide (LPS).
2. Incubate for 12-24 hours at 37° C in CO<sub>2</sub> incubator.
3. Test according to this protocol.

## **ASSAY PROCEDURE**

**Aseptic Procedures:** Steps 1 to 3 are aseptic procedures. Use sterile buffers and aseptic conditions, use laminar flow hood for procedures.

1. Wash 1 time with Cell Culture Media  
Fill each well completely with sterile Cell Culture Media. Don't discard until cells are ready to be plated.
2. Prepare Positive Control  
As described in **REAGENT PROVIDED**
3. Add 2 wells positive control, 2 wells negative control (unstimulated cells), 2 wells background control (sterile cell culture media) and GM-CSF secreting cells with appropriate concentration to each plate, 100 µL/well. Incubate at 37°C CO<sub>2</sub> incubator for 4-48 hours. Each researcher should determine the optimal incubation time based on the characteristics of the cell.

**Non-aseptic Procedures:** The following steps are non-aseptic procedures.

4. Prepare 1x Wash Buffer and Streptavidin – AP solution.  
As described in **REAGENT PROVIDED**.
5. Wash the plate 5 times with 1 x Wash Buffer  
Decant or aspirate contents of the plate into a waste container. Fill each well completely with 1 x Wash Buffer then decant or aspirate contents of the plate into a waste container. Repeat this procedure 4 more times for a total of 5 washes. After final wash, invert plate, and dry by hitting plate onto absorbent paper slightly.
6. Immediately add 100 µL of Human GM-CSF Detection Antibody to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
7. Repeat wash procedure as described in step 5. Wash plate 5 times.

8. Immediately add 100  $\mu$ L of Streptavidin-AP to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
9. Repeat wash procedure as described in step 5. Wash plate 5 times.
10. Immediately add 100  $\mu$ L of Substrate Solution to each well of the plate. Cover the plate and incubate 5-15 minutes at room temperature (20-25 °C) in dark.
11. Stop the assay  
Rinse 5 times with deionized water/distilled water. After final wash, invert plate, and dry by hitting plate onto absorbent paper slightly.
12. Dry plate  
Wet plates show higher background than completely dry plates. Remove the plastic underdrain from bottom of the plate. Allow the plate dry for 60-90 min at room temperature, or over night at room temperature, or 15-30 min at 37° C in dark. We recommend dry plate over night at room temperature.
13. Quantify spots using a dissection microscope or ELISpot reader.
14. Dried plate can be stored in sealed plastic bag in dark for 6 months.

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