A Novel Homogeneous Chemiluminescent Sandwich GM-CSF Assay Based on SPARCL™ Technology

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Abstract

A homogeneous, solution phase chemiluminescent immunoassay was developed* for the detection of GM-CSF based on SPARCL™ technology. This sandwich format immunoassay detects GM-CSF analyte with a capture antibody labeled with a chemiluminescent compound (acridan) and a complementary antibody labeled with HRP. In the presence of GM-CSF, HRP is brought into close proximity to the chemiluminescent compound, enabling light generation upon addition of a trigger solution (H_2O_2 and para-hydroxycinnamic acid, pHCA). There is no need to remove excess reagents prior to the detection step.

In a one-step, 30 minute incubation protocol utilizing 30uL of human serum an analytical sensitivity of 5 pg/mL was obtained. The dynamic range spanned five orders of magnitude.

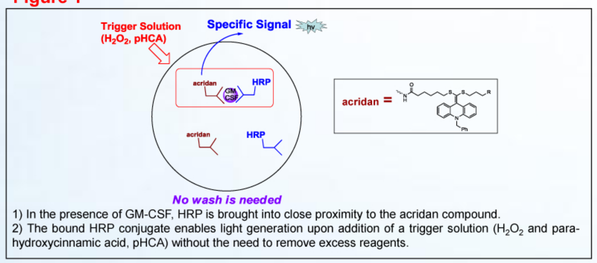
SPARCL GM-CSF assay offers significant advantages due to its simple no-wash/no-separation format, high sensitivity and ease of automation.

* Internal feasibility assay, not for commercial distribution

Introduction

GM-CSF, granulocyte macrophage colony stimulating factor, is a protein necessary for survival, proliferation and differentiation of hematopoietic progenitor cells[1]. A proximity dependent homogeneous chemiluminescent assay method enabling an efficient detection of GM-CSF was developed by completely eliminating wash steps based on SPARCL technology (Figure 1). This sandwich format immunoassay detects GM-CSF analyte with a capture antibody labeled with an acridan and a complementary antibody labeled with HRP (Format 1). Alternatively, the acridan can be attached to streptavidin and used in conjunction with a biotinylated capture antibody (Format 2).

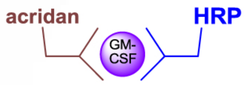
Figure 1



1) In the presence of GM-CSF, HRP is brought into close proximity to the acridan compound.
2) The bound HRP conjugate enables light generation upon addition of a trigger solution (H_2O_2 and para-hydroxycinnamic acid, pHCA) without the need to remove excess reagents.

GM-CSF Assay Format 1

Methods: To evaluate SPARCL in sandwich assay format, an anti-GM-CSF MAb was labeled with acridan and used with another, complementary, anti-GM-CSF MAb conjugated to HRP, see cartoon bellow (Format 1).



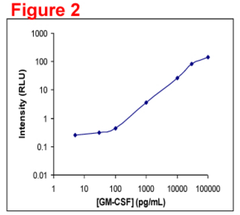
High-Volume Assay

Acridan labeled anti-GM-CSF capture antibody (30µL), anti-GM-CSF-HRP conjugate (30µL), GM-CSF calibrator (30µL), and human serum (30µL) were added into a 96-well white plate. After incubation for 30 minutes at room temperature, background reducer (2-aminophenol, 5µL) was added. Signal was generated by injecting 100µL of trigger solution and read for 5 seconds post triggering using a Labsystems Luminoskan 391** microplate luminometer.

Table 1

Conc (pg/mL)	Mean	S/S0
100000	141.9	839.3
30000	82.30	487.0
10000	25.68	152.0
1000	3.515	20.80
100	0.443	2.618
30	0.306	1.808
5	0.254	1.500
0	0.169	1.000

Figure 2



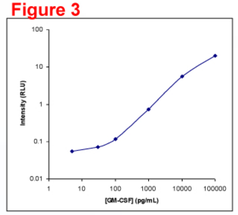
Low-Volume Assay

Acridan labeled anti-GM-CSF capture antibody (6µL), anti-GM-CSF-HRP conjugate (6µL), calibrator in human serum (6µL) were added into a 96-well white plate. After incubation for 30 minutes at room temperature, background reducer (2-aminophenol, 1µL) was added. Signal was generated by injecting 20µL of trigger solution and read for 2 seconds post triggering using same instrument as described above.

Table 2

Conc (pg/mL)	Mean	S/S0
100000	19.96	503.2
10000	5.639	142.1
1000	0.7433	18.74
100	0.1178	2.971
30	0.0713	1.798
5	0.0545	1.374
0	0.0397	1.000

Figure 3



Results: The Format 1 GM-CSF sandwich assays with a 30-minute incubation achieved a dynamic range of five logs. As shown in Figures 2 and 3, both volumes (low and high, as indicated previously) experimentally differentiated sample concentration of 5 pg/mL from the background.

GM-CSF Assay Format 2

Methods: To evaluate SPARCL in an alternative sandwich assay format, the acridan was attached to streptavidin and used in conjunction with a biotinylated anti-GM-CSF MAb. The complementary anti-GM-CSF MAb was labeled with HRP, see cartoon bellow (Format 2).

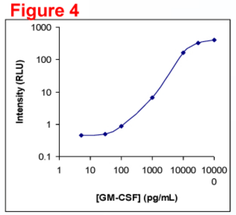
High-Volume Assay

Biotinylated anti-GM-CSF capture antibody (30µL), anti-GM-CSF-HRP conjugate in human serum (30µL), acridan labeled streptavidin (30µL) and GM-CSF calibrator (30µL) were added into a 96-well white plate. After incubation for 60 minutes at room temperature, background reducer (2-aminophenol, 5µL) was added. Signal was generated by injecting 100µL of trigger solution and read for 5 seconds post triggering using same instrument as described before.

Table 3

Conc (pg/mL)	Mean	S/S0
100000	399.8	1176
30000	321.1	944.4
10000	165.7	487.4
1000	6.5380	19.23
100	0.8810	2.591
30	0.5025	1.478
5	0.4460	1.312
0	0.3400	1.000

Figure 4



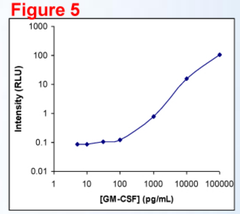
Low-Volume Assay

Biotinylated anti-GM-CSF capture antibody (6µL), anti-GM-CSF-HRP conjugate in human serum (6µL), acridan labeled streptavidin (6µL) and GM-CSF calibrator (6µL) were added into a 96-well white plate. After incubation for 60 minutes at room temperature, background reducer (2-aminophenol, 1µL) was added. Signal was generated by injecting 20µL of trigger solution and read for 2 seconds post triggering using same instrument as described before.

Table 4

Conc (pg/mL)	Mean	S/S0
100000	107.3	1883
10000	15.80	277.3
1000	0.7812	13.70
100	0.1232	2.161
30	0.1030	1.807
10	0.0878	1.541
5	0.0847	1.485
0	0.0570	1.000

Figure 5



Results: The Format 2 GM-CSF sandwich assays with a 60-minute incubation time achieved a dynamic range of five logs. As shown in Figures 4 and 5, both volumes (low and high, as indicated previously) experimentally differentiated sample concentration of 5 pg/mL from the background.

Conclusion

SPARCL GM-CSF assay provides an efficient solution phase assay method for the quantification of GM-CSF. In a one-step, 30 minute incubation protocol utilizing 30µL of human serum, sample concentration of 5 pg/mL was experimentally differentiated from the background (Figure 2). The dynamic range spanned five orders of magnitude (Figures 2-5). Both assay formats (Format 1 and 2) showed that assays volumes can be reduced without significantly affecting the detection limit. The added distinct advantages of SPARCL technology include no mixing or suspension of particles, and no wash steps which reduce cost and assay time. Moreover, complete solution phase resembles native biological environment and flash of signal reduces read time. SPARCL GM-CSF assay offers significant advantages due to its simple no-wash/no-separation format and high sensitivity.

References

1. Lawson D. J of Clinical Oncology 2000;8;1603-1605.