

High-Throughput Multiplexed Assays for Biomarkers of Hypoxia: VEGF, IGFBP-1 and Erythropoietin

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1 Abstract

Vascular Endothelial Growth Factor (VEGF) regulates angiogenesis; Insulin-like Growth Factor Binding Protein-1 (IGFBP-1) modulates the metabolic and mitogenic effects of IGFs; and Erythropoietin (EPO) regulates erythropoiesis. All three factors are upregulated by hypoxia. Electrochemiluminescence-based immunoassays were developed which allow simultaneous measurement of VEGF, IGFBP-1, and EPO in a 96-well format. The assay range is 10 pg/ml – 8,000 pg/ml for VEGF, 4 pg/ml – 40,000 pg/ml for IGFBP-1, and 0.4 mIU/ml – 2,000 mIU/ml for EPO. The assay protocol is simple: 25 μ L of sample and 25 μ L of detection antibody solution are incubated in the plate for 2 hours, followed by a wash step. Assays are performed in MSD 96-well 4-spot plates and are read on MSD's SECTOR™ Imager 6000, at a throughput of 80 samples per minute.

2 Introduction

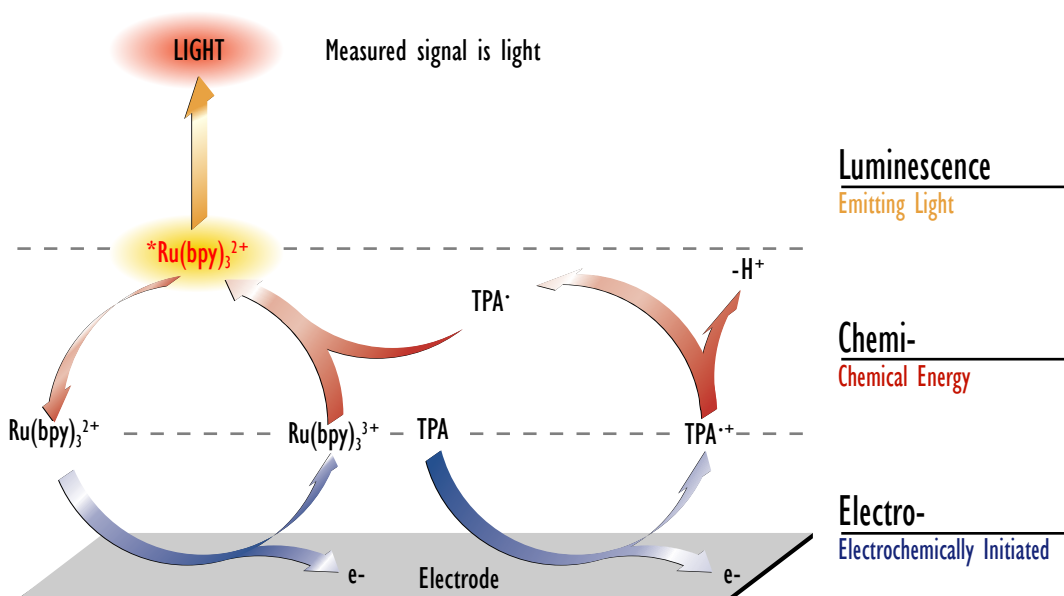
- Hypoxia: Lack of Oxygen
- Vascular Endothelial Growth Factor (VEGF) regulates angiogenesis
- Insulin-like Growth Factor Binding Protein-1 (IGFBP-1) modulates the metabolic and mitogenic effects of IGFs
- Erythropoietin (EPO) regulates erythropoiesis
- EPO, VEGF & IGFBP-1 are up-regulated by hypoxia
- Intended use of the assay: measure EPO, VEGF & IGFBP-1 concentrations in cell culture supernatant of hypoxic & normoxic cells in a high-throughput format
- All three analytes are measured simultaneously in a 96-well 4-spot plate



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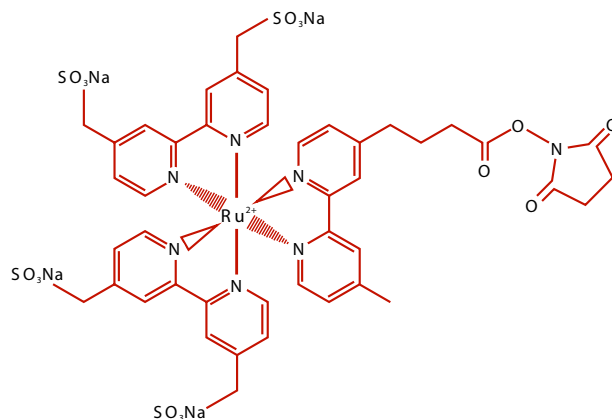
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3 Electrochemiluminescence (ECL)



Ruthenium (II) tris-bipyridine-(4-methylsulfone) NHS ester (MSD SULFO-TAG™ label)

- Selective
- Convenient chemistry
- Robust, stable
- Few interferences



- Size, MW: ~1200 daltons
- Stability: Years
- Solubility: Aqueous, DMSO
- Functionality: Hydrophilic
- Specificity: High



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4 Meso Scale Discovery MULTI-ARRAY™ Technology

Instrument Features

- Highly sensitive
- SECTOR Imager designed for high-throughput screening (HTS)
- SECTOR Imager or SECTOR PR Reader ideal for assay development
- Custom optics
- High-speed motion control systems
- Electrochemiluminescent (ECL) detection



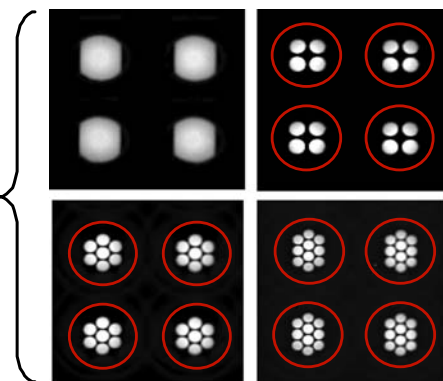
SECTOR PR™ Reader



SECTOR™ Imager 6000

Plate Features

- Disposable Plates
- Carbon Electrodes with high binding capacity
- Suitable electrochemistry for ECL
- Biocompatible: direct immobilization of avidin, IgG, membrane fragments, intact cells, etc.
- Functional Assays: simple binding reactions, GPCRs, enzyme cascades, post-translational modification, etc.



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5 Assay Format

- Block plates for one hour at RT or overnight at 4°C and wash (optional)
- Add 25 µl each of detection AB reagent and sample to each well
- Incubate for two hours on a shaker
- Wash, add read buffer, read

6 Alternative Assay Formats

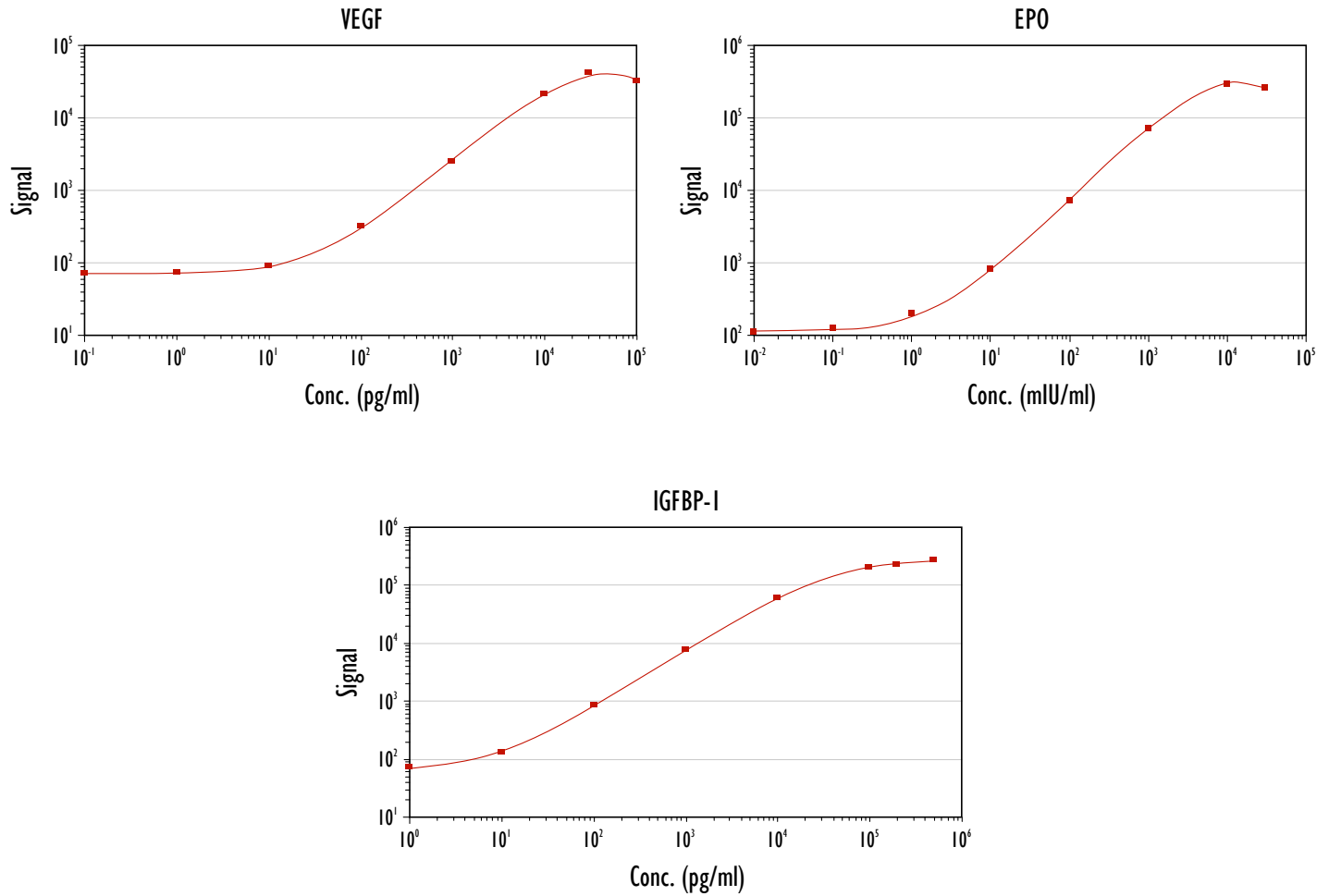
- Standard assay format: block, wash, 2-hour incubation, wash, read
- Alternative I: omit wash step before read: 2-5x higher detection limits
- Alternative II: omit wash step before read; omit blocking step: 5-8x higher detection limits
- Alternative III: 2-hour incubation with sample, wash, 1 hour incubation with detection antibody, wash, read: no hook effect; 10x larger dynamic range



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7 Calibration Curve: VEGF, EPO and IGFBP-1



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8 Sensitivity & Dynamic Range

Analytical sensitivity: analyte concentration at background plus 2.5 SD of background.

16 replicates of zero calibrator were run. Average counts and SD (counts) was calculated. Signal at average zero counts plus 2.5 SD was transformed in analyte concentration based on the 4-parameter calibration curve.

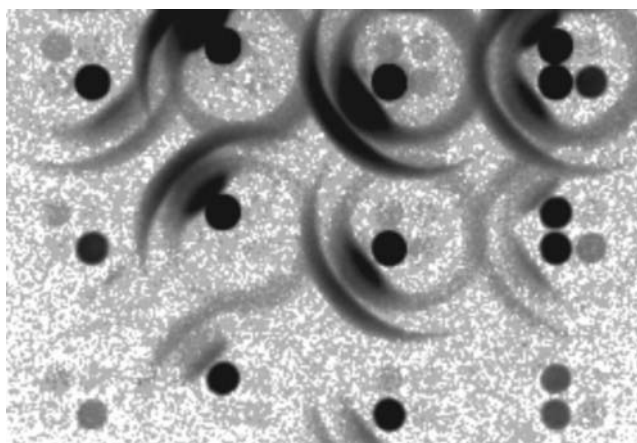
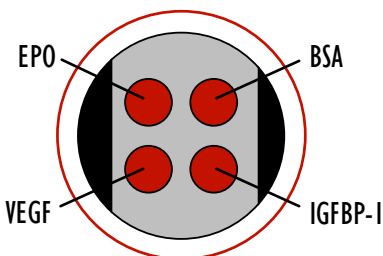
	Average Counts at zero calibrator	SD (counts)	Analytical sensitivity
VEGF	68	9	10 pg/ml
IGFBP-1	63	12	4 pg/ml
EPO	121	9	0.4 mIU/ml

	Analytical Sensitivity	Upper End of Assay Range	Hook Concentration	Dynamic Range
VEGF	10 pg/ml	8,000 pg/ml	30,000 pg/ml	800
IGFBP-1	4 pg/ml	40,000 pg/ml	500,000 pg/ml	10,000
EPO	0.4 mIU/ml	2,000 mIU/ml	10,000 mIU/ml	5,000



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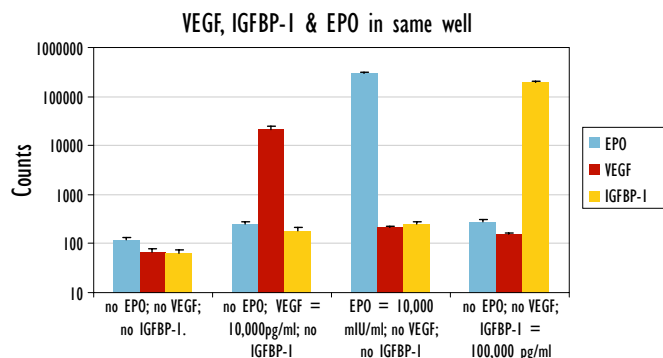
9 EPO — VEGF — IGFBP-1 Multiplexing



% Cross-reactivity

	EPO	VEGF	IGFBP-1
EPO	x	0.64%	0.08%
VEGF	0.05%	x	0.05%
IGFBP-1	0.07%	0.57%	x

% Cross-reactivity: (signal in presence of interferent - signal in absence of interferent)/interferent signal



10 Conclusion

An assay was developed to simultaneously measure EPO, VEGF, and IGFBP-1 in a high-throughput format (96-well 4-spot plate; simple assay protocol; 80 samples/minute throughput).

Cross-reactivity is less than 1%.

Assay ranges are 10 pg/ml — 8,000 pg/ml for VEGF, 4 pg/ml — 40,000 pg/ml for IGFBP-1, and 0.4 mIU/ml — 2,000 mIU/ml for EPO.



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