A Comparison of AlphaLISA Bead-Based Luminescence and Electrochemiluminescence Immunoassay Technologies for Detection of Human EPO, Amyloid Beta 42 and VEGF in Complex Sample Matrices

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1 Abstract

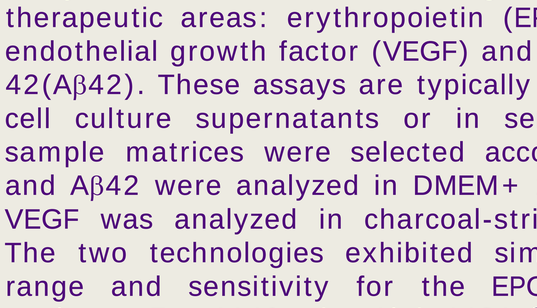
Robust, sensitive and reproducible immunoassays for biomolecules are essential for the drug discovery process. When scaled up for high throughput screening, assay complexity, automation and cost become critical. Ideally, an assay should be amenable to scale-up without any compromise to performance.

We compared two assay platforms commonly used for drug discovery and examined performance (sensitivity, dynamic range, variability) as well as assay complexity, time to perform, and cost. The AlphaLISA assay kits, EnVision® Multidetection Reader, and microplates were supplied by PerkinElmer. The electrochemiluminescence (ECL) kits, dedicated ECL reader and microplates were provided by an alternative supplier. The three analytes tested cover a range of different therapeutic areas: erythropoietin (EPO), vascular endothelial growth factor (VEGF) and amyloid beta 42(Aβ42). These assays are typically performed in cell culture supernatants or in serum, so the sample matrices were selected accordingly. EPO and Aβ42 were analyzed in DMEM+ 1% FBS, and VEGF was analyzed in charcoal-stripped serum. The two technologies exhibited similar dynamic range and sensitivity for the EPO and VEGF analytes. For Aβ42, the AlphaLISA ® assay gave slightly higher upper and lower detection limits (UDL and LDL), but the overall dynamic range was similar for the two assays. Percent recovery values were determined for the EPO assays, and both assay technologies showed low variability and good accuracy.

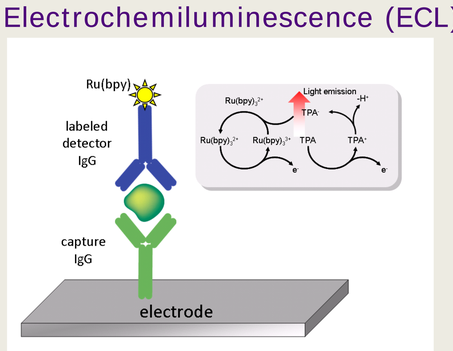
The AlphaLISA assay employed a faster and less complex assay protocol that was more amenable to automation due to the lack of wash steps. For EPO, the AlphaLISA also consumed five times less sample volume & considerably less time to achieve the same sensitivity. These process benefits, combined with the generally lower cost of AlphaLISA reagents and instrumentation, make the AlphaLISA assay platform particularly attractive for high throughput screening applications.

2 Introduction

AlphaLISA Technology



Electrochemiluminescence (ECL) Technology



3 Materials & Methods

Plates and equipement
AlphaLISA: OptiPlate™-384 white (PerkinElmer, #6007299)
EnVision Multilabel Plate Reader

Matrix components (DMEM F12 + 1% Hi FBS)
DMEM F12 (Invitrogen, #11039-021)
Heat-inactivated FBS (Wisent, #080450)
Pooled Charcoal Stripped Human Serum, (Innovative Research #IPA-SER6)

Kits
hEPO AlphaLISA kit (AL206C)
hVEGF AlphaLISA kit (AL201C)
hAmyloid beta 1-42 AlphaLISA kit (AL203C)

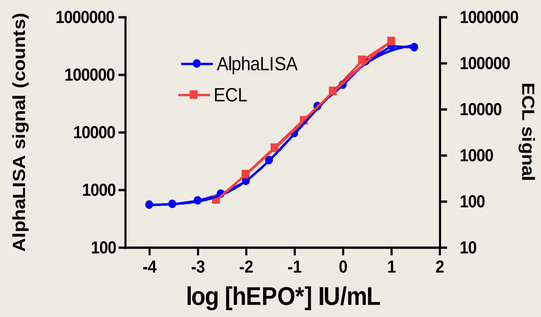
Representative AlphaLISA Protocol

1. Add standards/samples: 20 µL for VEGF and Aβ42, 5 ul for EPO.
2. Add 5µL Anti-biomarker IgG#1 coated Acceptor beads
3. Incubate 30 minutes
4. Add 5µL biotinylated Anti-biomarker IgG#2
5. Incubate 60 minutes
6. Add 20 µL SA-Donor beads
7. Incubate 30 minutes in the dark
8. Read plate on Envision

Representative ECL Protocol Performed by the University of Kansas

1. Add 150µL of blocker C
2. Incubate 1-2 hours on the shaker at 700 rpm
3. Wash plate 3X
4. Add 25µL Assay diluent
5. Add 25µL standards/samples
6. Incubate 2 hours at 700 rpm
7. Wash 3X
8. Add 25µL SULFO-TAG Anti-biomarker IgG
9. Incubate 2 hours at 700 rpm
10. Wash 3X
11. Add 150µL Read Buffer
12. Read plate on dedicated ECL plate reader

4 hEPO



*DMEM+1% FBS

hEPO satndard curves in complex matrix.
Using their respective and recommended protocols, AlphaLISA and ECL **show similar sensitivities** (lower detection limit defined as LDL= mean of background value + 3SD).

Range	AlphaLISA	ECL
Minimum	1 mIU/ml	1 mIU/ml
Maximum	10,000 mIU/ml	10,000 mIU/ml
Total	5 Log	5 Log

AlphaLISA and ECL have **similar ranges**, though ECL requires larger sample volume.

Recovery:
AlphaLISA spike-in concentrations: 3, 30 and 3000 mIU/ml.
ECL spike-in concentrations: 50, 500 and 1000 mIU/ml.

Spiking	Concentrations	AlphaLISA	ECL
% recovery spike-in	High	83	132
	Medium	94	118
	Low	98	112
% recovery competitor spike-in	High	63	140
	Medium	70	135
	Low	70	140

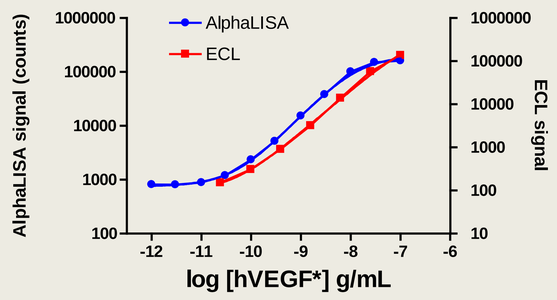
AlphaLISA and ECL show **suitable recoveries** when used with their respective standard analytes.

Precision:
Intra-assay: 9 replicates, Inter-assay: 3 x 9 replicates

% CV	Concentrations	AlphaLISA	ECL
Intra-assay precision	High	2.44	2.45
	Medium	3.28	2.27
	Low	3.21	5.87
Inter-assay precision	High	5.83	5.41
	Medium	5.57	6.51
	Low	11.20	8.65

AlphaLISA and ECL have **similar intra- and inter-assay precision** at all tested concentrations.

5 hVEGF



*in stripped serum

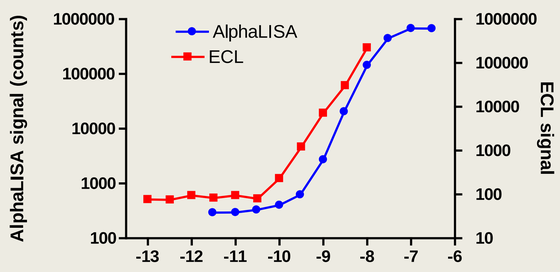
hVEGF standard curves in complex matrix.
Using their respective and recommended protocols, AlphaLISA and ECL **show similar sensitivities** (lower detection limit defined as LDL= mean of background value + 3SD).

Linear dynamic range:

Range	AlphaLISA	ECL
Minimum	9 pg/mL	8 pg/mL
Maximum	100 ng/mL	100 ng/mL
Total	4 Log	4 Log

AlphaLISA and ECL have **similar ranges**, though ECL requires slightly larger sample volume.

6 hAβ42



*in DMEM+ 1% FBS

hAβ42 standard curves in complex matrix.
Using their respective and recommended protocols, ECL showed slightly higher sensitivity (5 fold) than AlphaLISA. (lower detection limit defined as LDL= mean of background value + 3SD).

Linear dynamic range:

Range	AlphaLISA (Two-step protocol)	ECL
Minimum	235 pg/mL	50 pg/mL
Maximum	300 ng/mL	100 ng/mL
Total	3 Log	3 Log

AlphaLISA and ECL have **similar ranges**, though ECL requires slightly larger sample volume..

7 Summary

For the detection of three biomarkers in complex sample matrices, the AlphaLISA and Electrochemiluminescent (ECL) assay technologies were shown to have similar:
- Assay windows (linear dynamic range),
- Lower and upper detection limit,
- Intra- and inter-assay precision (lower %CV)

The advantages of using AlphaLISA over ECL are:
- Shorter total assay duration
- No wash steps
- No shaking
- Lower sample volume requirement for equivalent performance
- Less expensive instrument and plates required

In this study three AlphaLISA no-wash assays, which employ a faster and less complex assay protocol, were found to deliver highly sensitive and accurate results, equivalent to those obtained with the ECL technology.

The High Throughput Screening Laboratory at the University of Kansas is funded in part by NIH/NCRR COBRE grant P20 RR015563 (PI, Dr. Barbara Timmermann).

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