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(A4/42). These assays are typically performed in cell culture supernatants or in serum, so the sample matrices were selected accordingly. EPO and A4/42 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 A4/42, 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.

Introduction

AlphaLISA Technology

Electrochemiluminescence (ECL) Technology

Materials & Methods

Plates and equipment

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

Matrix components (DMEM F12 + 1% FBS)
DMEM F12 (Invitrogen, #11330-021)
Heat-inactivated FBS (Wisent, #980450)
Pooled Charcoal Stripped Human Serum, (Innovative Research #IPA-ERK)

Kits

hEPO AlphaLISA kit (AL206C)
HVEGF AlphaLISA kit (AL201C)
hA4/42 AlphaLISA kit (AL203C)

Representative AlphaLISA Protocol

1. Add 50µl 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 20 µL SA-Donor beads
9. Incubate 60 minutes
10. 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 antibody
5. Add 25µl standards/samples
6. Incubate 2 hours at 700 rpm
7. Wash 3X
8. Add 20 µL SA-Donor beads
9. Incubate 60 minutes
10. Read plate on dedicated ECL plate reader

hEPO

DMEM+1% FBS

αEPO signal (Counts)

Linear dynamic range:

Minimum 235 pg/mL 50 pg/mL
Maximum 10,000 mIU/mL 10,000 mIU/mL
Total 4 Log 4 Log

Comparison of AlphaLISA and ECL

AlphaLISA and ECL have similar sensitivities (lower detection limit defined as LDL= mean of background value + 3SD).

<table>
<thead>
<tr>
<th>Range</th>
<th>AlphaLISA</th>
<th>ECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1 mIU/mL</td>
<td>1 mIU/mL</td>
</tr>
<tr>
<td>Maximum</td>
<td>10,000 mIU/mL</td>
<td>10,000 mIU/mL</td>
</tr>
<tr>
<td>Total</td>
<td>5 Log</td>
<td>5 Log</td>
</tr>
</tbody>
</table>

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

Recovery

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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>AlphaLISA</th>
<th>ECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% recovery spike-in</td>
<td>High</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>98</td>
</tr>
<tr>
<td>% recovery spike-in</td>
<td>High</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>70</td>
</tr>
</tbody>
</table>

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

Precision

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

<table>
<thead>
<tr>
<th>% CV</th>
<th>Concentrations</th>
<th>AlphaLISA</th>
<th>ECL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-assay precision</td>
<td>High</td>
<td>3.28</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>3.21</td>
<td>5.87</td>
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<tr>
<td></td>
<td>Low</td>
<td>5.83</td>
<td>5.41</td>
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<tr>
<td>Inter-assay precision</td>
<td>High</td>
<td>5.57</td>
<td>6.51</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>11.20</td>
<td>8.65</td>
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</tbody>
</table>

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

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 limits,
• 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 NH/NCRR COBRE grant P20 RR015563 (PI, Dr. Barbara Timmermann).