All-In-One-Well AlphaLISA Assays for Direct Biomarker Quantification: Cell Cultures

Robertino Rodriguez-Suarez, Marie-Hélène Venne, Nancy Mc Donald, Marjolaine Roy, Chantal Illy and Stéphane Parent

Abstract

The used of cultures for the assessment of compound activity offers many advantages over in vitro biochemical assays. Cellular models allow the simultaneous evaluation of various signaling pathways and the evaluation of a drug’s permeability to cellular membranes. An End-Point Assay using Enzyme-Linked Immunosorbent Assays (ELISA) is the most widely adopted method for biomarker detection and quantification. ELISA offers high selectivity, sensitivity and assay versatility; however, it presents certain limitations such as a narrow dynamic range, low throughput and modest reproducibility due to its numerous wash steps. In contrast, homogeneous chemiluminescent bead AlphaLISA® assays allow measurement of biomarkers in a high throughput mode in the absence of any wash step. In the present work, various cytokines (TNF, IL1, IL6, IL8) and an integral plasma membrane protein (ECD) were measured on stimulated cells using an All-In-One-Well AlphaLISA® assay format. Biomarker production was measured from the adherent cell lines A431 or A549, and from suspension THP-1 cells directly in 384-well culture plates, in the absence of any transfer or wash step. This technology simplifies cellular assays, significantly reduces hand-on-time and costs associated with plastic ware, and improves reproducibility. Moreover, results show excellent assay performance with wide dynamic range, true interference from cells or cell culture media, and high sensitivity. Indeed, AlphaLISA technology is a user-friendly and versatile tool for generating immunoassays for cellular models.

Introduction

AlphaLISA® assay

The Streptavidin-coated Alpha Donor bead (Star) is used to label the streptavidin-conjugated analyte antibody. The AlphaLISA® Acceptor bead (red) is coated with anti- specific antibody. The analyte is captured by the Acceptor bead through antibody binding. The Star bead is excited by a 635 nm laser, and the resulting radiation is captured by the AlphaLISA® detector. The emitted light travels to the nearby AlphaLISA® detector, where the signal is amplified and converted into a countable number.

Materials & Methods

AlphaLISA® kits

Equipment

Enzyme-Linked Multiplate Reader

Produced by:

AlphaLISA® assay

Materials & Methods

1. For the standard curves, TNFα was spiked at different concentrations in wells containing only medium or was stimulated THP-1 cells, and B) cells in log phase were plated and stimulated with different concentrations of LPS. All AlphaLISA® reagents were added to the same well for the detection and TNFα concentrations were determined from the standard curve.

2. The LPS-induced TNFα was efficiently detected by AlphaLISA® in a single well format.

3. The assay was not affected by the presence of cells.

4. Although RPMI contains free biotin, a good sensitivity was obtained.

5. Interleukin 1 beta

6. Interleukin 6

7. Interleukin 8

8. Epidermal Growth Factor Receptor

9. Summary

- Biomarker levels were measured directly in CulturPlates and -384 in a single, fast, all-in-one-Well AlphaLISA® assay format (also compatible with 1536-well plates).
- The elimination of transfer and wash steps simplifies cellular assays, reduces variability, and significantly reduces hand-on-time and costs associated with consumables.
- Introducing immobilized receptor- and associated soluble proteins (TNFα, IL1, IL6, IL8) were successfully detected, allowing rapid receptor detection, using the standard AlphaLISA® Immunoassay buffer.
- Excellent dynamic range, low interference from cells or cell culture media (e.g. DMEM, 10% FBS), high reproducibility and sensitivity.

Epidermal Growth Factor Receptor

EGFR measurement. A) The cells (A431 and HEK293) were plated at different concentrations and incubated 1h at 37°C. 5% CO2, then the cells were washed twice with PBS and or AlphaLISA® Immunoassay buffer was added to lys the cells for 10 minutes at 23°C. The cell medium were then placed in the same well. B) The cells (A431 and HEK293) were plated at different concentrations in medium containing either 1% or 2% Nonidet P-40. Then, the cells were washed twice with PBS after which AlphaLISA® Immunoassay buffer with or without Triton X-100 (1%) was added for 10 minutes at 23°C. The AlphaLISA® EGFR assay was then performed in the same well.

- EGFR can be detected in as few as 40 A431 or 1600 HEK293 plated cells. This difference is in line with the -50-fold difference in EGFR expression levels reported for these two cell types (approximately 1:20; Genentech, reports of 1997, PMAD).

- No significant difference was found between PBS-washed and non-washed cells after 18h of plating (without any interference from the cell culture media). However, at longer incubation times, the contribution of secreted EGFR-EC in A431 cells should be evaluated.

- The use of an Immunoassay buffer without Triton X-100 proved to have a marked effect on assay sensitivity, causing a 30-fold and 15-fold drop in the signal with the A431 and HEK293 cells respectively, showing that the EGFR protein is detected inside the membrane-bound form.

The LPS-induced IL6 was efficiently detected by AlphaLISA® in a single well format.

The assay was not affected by the presence of cells.

Although RPMI contains free biotin, a good sensitivity was obtained.

Induced IL8 was efficiently detected by AlphaLISA® in a single well in both THP-1 and A459 cell lines.

Signal in the presence of non-stimulated THP-1 or A549 cells is likely due to significant IL8 basal levels. IL8 can be detected in IL8-stimulated A459 cells using as few as 100 cells/well.

Effect of Triton X-100

0 10,000 20,000 30,000
100
1,000
10,000
100,000
1,000,000 with Triton
no Triton
with Triton
no Triton
A431HEK293
seeded cells (#)

<table>
<thead>
<tr>
<th>Condition</th>
<th>no Triton</th>
<th>with Triton</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431 seeded cells</td>
<td>100,000</td>
<td>100,000</td>
</tr>
<tr>
<td>HEK293 seeded cells</td>
<td>100,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>

The LPS-induced IL8 was efficiently detected by AlphaLISA® in a single well format.

The assay was not affected by the presence of cells.

Although RPMI contains free biotin, a good sensitivity was obtained.

EGF measurement. A) The cells (A431 and HEK293) were plated at different concentrations and incubated 1h at 37°C. 5% CO2, then the cells were washed twice with PBS and or AlphaLISA® Immunoassay buffer was added to lys the cells for 10 minutes at 23°C. The cell medium were then placed in the same well. B) The cells (A431 and HEK293) were plated at different concentrations in medium containing either 1% or 2% Nonidet P-40. Then, the cells were washed twice with PBS after which AlphaLISA® Immunoassay buffer with or without Triton X-100 (1%) was added for 10 minutes at 23°C. The AlphaLISA® EGFR assay was then performed in the same well.

- EGFR can be detected in as few as 40 A431 or 1600 HEK293 plated cells. This difference is in line with the -50-fold difference in EGFR expression levels reported for these two cell types (approximately 1:20; Genentech, reports of 1997, PMAD).

- No significant difference was found between PBS-washed and non-washed cells after 18h of plating (without any interference from the cell culture media). However, at longer incubation times, the contribution of secreted EGFR-EC in A431 cells should be evaluated.

- The use of an Immunoassay buffer without Triton X-100 proved to have a marked effect on assay sensitivity, causing a 30-fold and 15-fold drop in the signal with the A431 and HEK293 cells respectively, showing that the EGFR protein is detected inside the membrane-bound form.

The LPS-induced IL6 was efficiently detected by AlphaLISA® in a single well format.

The assay was not affected by the presence of cells.

Although RPMI contains free biotin, a good sensitivity was obtained.

Induced IL8 was efficiently detected by AlphaLISA® in a single well in both THP-1 and A459 cell lines.

Signal in the presence of non-stimulated THP-1 or A549 cells is likely due to significant IL8 basal levels. IL8 can be detected in IL8-stimulated A459 cells using as few as 100 cells/well.

Effect of Triton X-100

0 10,000 20,000 30,000
100
1,000
10,000
100,000
1,000,000 with Triton
no Triton
with Triton
no Triton
A431HEK293
seeded cells (#)

<table>
<thead>
<tr>
<th>Condition</th>
<th>no Triton</th>
<th>with Triton</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431 seeded cells</td>
<td>100,000</td>
<td>100,000</td>
</tr>
<tr>
<td>HEK293 seeded cells</td>
<td>100,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>

The LPS-induced IL8 was efficiently detected by AlphaLISA® in a single well format.

The assay was not affected by the presence of cells.

Although RPMI contains free biotin, a good sensitivity was obtained.

Induced IL8 was efficiently detected by AlphaLISA® in a single well in both THP-1 and A459 cell lines.

Signal in the presence of non-stimulated THP-1 or A549 cells is likely due to significant IL8 basal levels. IL8 can be detected in IL8-stimulated A459 cells using as few as 100 cells/well.

Effect of Triton X-100

0 10,000 20,000 30,000
100
1,000
10,000
100,000
1,000,000 with Triton
no Triton
with Triton
no Triton
A431HEK293
seeded cells (#)

<table>
<thead>
<tr>
<th>Condition</th>
<th>no Triton</th>
<th>with Triton</th>
</tr>
</thead>
<tbody>
<tr>
<td>A431 seeded cells</td>
<td>100,000</td>
<td>100,000</td>
</tr>
<tr>
<td>HEK293 seeded cells</td>
<td>100,000</td>
<td>100,000</td>
</tr>
</tbody>
</table>

The LPS-induced IL8 was efficiently detected by AlphaLISA® in a single well format.

The assay was not affected by the presence of cells.

Although RPMI contains free biotin, a good sensitivity was obtained.

Induced IL8 was efficiently detected by AlphaLISA® in a single well in both THP-1 and A459 cell lines.

Signal in the presence of non-stimulated THP-1 or A549 cells is likely due to significant IL8 basal levels. IL8 can be detected in IL8-stimulated A459 cells using as few as 100 cells/well.