Lot-to-Lot Comparison of Streptavidin-PE Conjugates in Two Commercial Assays

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Abstract
The properties of the conjugate incorporating the reporter dye (streptavidin-PE) impact assay performance to a great degree. The optimal conjugate is bright, exhibits minimal non-specific binding and demonstrates consistency lot to lot. Three lots of ProZyme’s PhycoLink® PJ31S were compared in two commercial assays.

Introduction
Phycoerythrin (PE) was chosen as the reporter dye for xMAP® Technology because of its excellent performance in flow applications; its extremely high molar absorptivity and quantum efficiency make it the brightest fluor available with the desired spectral properties. However, the properties of the streptavidin-PE conjugate also impact assay performance to a significant degree.

Characteristics of the optimal conjugate:
- highest level of fluor per binding event (brightness)
- minimal background (non-specific binding)
- consistency lot to lot

Two commercial assays for Human IL-4 and IFN-γ were compared; PJ31S demonstrated the highest signal for the series of conjugates tested (data not shown). Three lots of PJ31S were then compared for lot-to-lot consistency.

Results

Streptavidin-PE Conjugates Available from ProZyme
ProZyme has worked with a number of partners to optimize streptavidin-PE conjugates for specific assays. Results for a single lot are not consistent with different assay formats (e.g. nucleic acid vs. protein analytes), suggesting that the optimal conjugate differs across applications (data not shown). Streptavidin and PE were conjugated under controlled conditions to produce a series of products with improved performance for specific assays.

PJ31S
The largest, brightest conjugate. Provides the highest fluorescence for a biotin-binding event.

PJL5S
Similar to PJ31S, but made with a proprietary Phycoerythrin (slightly different spectral properties).

PJ33S
Different molar ratio conjugate, works well in systems with excess biotin.

PJ55S
A smaller conjugate than PJ31S or PJL5S. May demonstrate lower background in some applications.

PJ77S
Smaller than PJ35S.

PJ99S
The most defined composition conjugate. Monomeric, when characterized by HPLC. For applications requiring consistent signal per binding event, and where maximum brightness is not a requirement.

Lot-specific Information:

<table>
<thead>
<tr>
<th>Product Code</th>
<th>PJ31S</th>
<th>PJL5S</th>
<th>PJ33S</th>
<th>PJ55S</th>
<th>PJ77S</th>
<th>PJ99S</th>
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<tbody>
<tr>
<td>Lot Number</td>
<td>841 049</td>
<td>841 055</td>
<td>841 063</td>
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<tr>
<td>Concentration (mg/ml)</td>
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<td>2.09</td>
<td>2.13</td>
<td>2.03</td>
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<tr>
<td>PE molarity (μM)</td>
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<td>Streptavidin molarity (μM)</td>
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<td>10.1</td>
<td>11.0</td>
<td>10.0</td>
<td>10.0</td>
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<tr>
<td>% binding of A&lt;sub&gt;max&lt;/sub&gt; to biotin-agarose</td>
<td>100</td>
<td>100</td>
<td>&gt;90%</td>
<td>99</td>
<td>98</td>
<td>80</td>
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<td>A&lt;sub&gt;max&lt;/sub&gt;/A&lt;sub&gt;sp&lt;/sub&gt;</td>
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<td>2.1</td>
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<td>3.6</td>
<td>4.2</td>
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<td>&gt;500</td>
<td>&gt;440</td>
<td>&gt;350</td>
<td>&gt;350</td>
<td>&gt;290</td>
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</table>

Conclusion
- Choose the optimal conjugate for the assay format from a series of ProZyme’s streptavidin-PE conjugates in order to optimize performance.

Request the PhycoLink® SA-PE Sampler Kit (product code PJ3XS)
- Count on consistent performance (due to ProZyme’s controlled processes) from commercially available conjugates.

Methods
Standard curves were prepared using the Bio-Plex™ Cytokine Reagent Kit (product code 171-304000) with a mixture of Human IL-4 (171B10452) and IFN-γ (171B11921) assays to compare lots of streptavidin-PE conjugates using a Bio-Plex™ 200 instrument running the Bio-Plex Manager™ Software v4.1. The 17-Plex Human Cytokine Standard was serially diluted to a set of 8 dilutions: from 3,200 pg/ml, to 800 pg/ml, 200 pg/ml, 50 pg/ml, 12.5 pg/ml, 3.12 pg/ml, 0.781 pg/ml and 0.195 pg/ml. Each conjugate was tested with the bead mixture, the Human Cytokine Standard dilution series, and the two assays’ biotinylated detection antibodies. The data were plotted on a log-log chart.

R-Phycoerythrin Absorbance and Fluorescence Emission Spectra

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