

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

Archie Chen and  
Tim Legerton



## 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

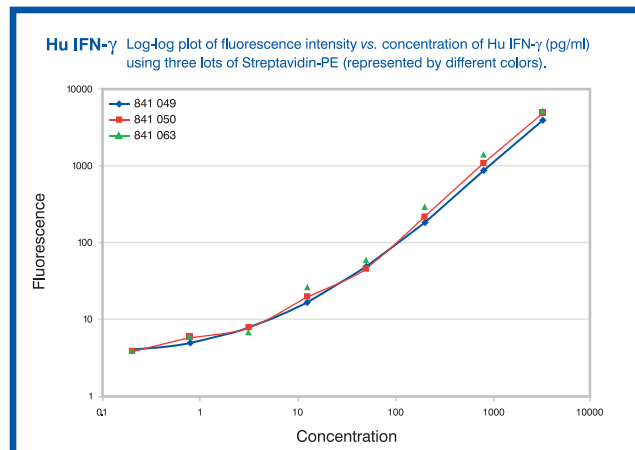
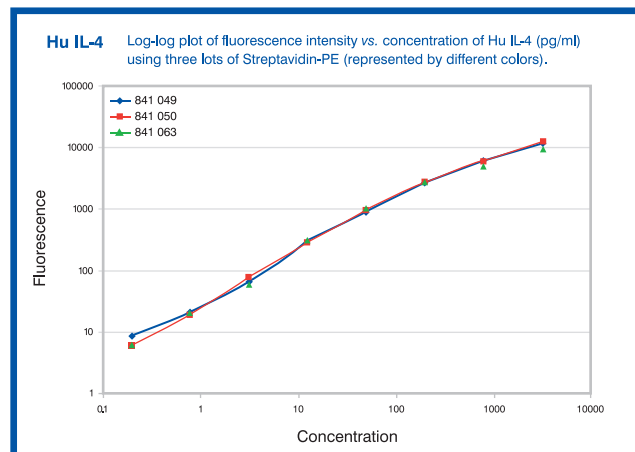
Phycoerythrin (PE) was chosen as the reporter dye for xMAP® Technology because of its excellent performance in flow applications; its extremely high molar absorptivity and quantum efficiency make it the brightest fluor available with the desired spectral properties. However, the properties of the streptavidin-PE 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- $\gamma$  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



### Lot-specific Information:

Product Code	PJ31S	PJ31S	PJ31S
Lot Number	841 049	841 050	841 063
Concentration (mg/ml)	2.14	2.09	2.13
PE molarity ( $\mu\text{M}$ )	6.5	6.4	6.4
Streptavidin molarity ( $\mu\text{M}$ )	10.3	10.1	11.0
% binding of $A_{\lambda_{\text{max}}}$ to biotin-agarose	100	100	100
$A_{\lambda_{\text{max}}}/A_{280}$	3.0	3.0	2.9
Apparent molecular weight (kDa, size exclusion chromatography)	$\geq 500$	$\geq 500$	$\geq 500$

## 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- $\gamma$  (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.

## 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.

<b>PJ31S</b>	The largest, brightest conjugate. Provides the highest fluorescence for a biotin-binding event.
<b>PJLS</b>	Similar to PJ31S, but made with a proprietary Phycoerythrin (slightly different spectral properties).
<b>PJ33S</b>	Different molar ratio conjugate; works well in systems with excess biotin.
<b>PJ35S</b>	A smaller conjugate than PJ31S or PJLS. May demonstrate lower background in some applications.
<b>PJ37S</b>	Smaller than PJ35S.
<b>PJ39S</b>	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:

Product Code	PJ31S	PJLS	PJ33S	PJ35S	PJ37S	PJ39S
Lot Number	841 050	DG88 009	DE29 147	DE29 064	DE29 092	DE29 093
Concentration (mg/ml)	2.09	2.17	0.69	2.12	2.12	2.06
PE molarity ( $\mu\text{M}$ )	6.4	6.3	1.6	7.3	7.2	7.6
Streptavidin molarity ( $\mu\text{M}$ )	10.1	11.8	5.9	7.0	7.0	4.2
% binding of $A_{\lambda_{\text{max}}}$ to biotin-agarose	100	100	$\geq 90\%$	99	98	80
$A_{\lambda_{\text{max}}}/A_{280}$	3.0	3.1	2.1	3.6	3.6	4.2
Apparent molecular weight (kDa, size exclusion chromatography)	$\geq 500$	$\geq 500$	$\geq 440$	$\geq 350$	$\geq 350$	$\sim 290$

## 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.

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## R-Phycoerythrin Absorbance and Fluorescence Emission Spectra

