

MULTIPLE-LOT COMPARISON OF PHYCOLINK® PJ25S STREPTAVIDIN-ALLOPHYCOCYANIN CONJUGATES IN A PERFORMANCE ASSAY

Allophycocyanin (APC) is widely used as a reagent for screening assays, where it performs the role of acceptor molecule in fluorescent resonance energy transfer (FRET) assays. For laboratories employing such assays, consistent performance and brightness of APC conjugates has become an issue of increasing importance as these assays move into the screening phase, involving large numbers of individual determinations.

The most important characteristic of any reagent intended for use in a FRET assay is consistent performance in that assay. No other measurable characteristic of a reagent can provide as complete an assurance of performance. For this reason, ProZyme works with customers to develop specific performance tests for its reagents in major applications.

The most commonly used APC reagent is Streptavidin-APC (SA-APC), and ProZyme began its development of APC performance tests with this reagent. As a primary manufacturer of both APC and SA, ProZyme is in a unique position to provide SA-APC conjugates of the highest possible quality and consistency, as clearly demonstrated by the results presented here.

PROCEDURES

Four consecutive production lots (lots #896 042, 896 043, 896 044 and 896 045) of PhycoLink SA-APC Conjugate (Cat# PJ25S)

were tested for performance in a TR- FRET assay with biotinylated-Europium chelate [Wallac #Eu-W1024] (B-Eu) acting as the fluorescence donor. Characteristics reported on the Certificate of Analysis for each lot are reported in Table 1.

Lot #	896 042	896 043	896 044	896 045
μ M APC	12.3	12.2	12.2	12.4
μ M SA	13.3	14.4	14.3	13.9
APC/SA	92.5	84.7	85.3	89.2
% binding*	99.4	99.4	99.6	99.9

* % A_{650} that binds to a biotin-agarose column

Each lot was assayed independently four times. In all cases, SA-APC at a single concentration of ~ 0.2 nM was dispensed into 96-well plates and titrated in duplicate with 0.2 to 1.8 nM B-Eu to determine the maximum signal achievable when available SA binding sites were saturated. After mixing, plates were read at 10 min, 1.5 h, 18 h and 24 h on a Wallac Victor² 1420 Multilabel Counter (PerkinElmer) with the Lance™ 665/615 protocol, which excites the Eu donor at 340 nm and reads fluorescence at 615 nm (direct Eu fluorescence) and 665 nm (FRET to APC).

RESULTS

Lot-to-lot comparisons provide an indicator of conjugate consistency with respect to both SA binding levels and APC fluorescence. No significant differences were observed in the 665 nm signals obtained with different lots of SA-APC (Figure 1).

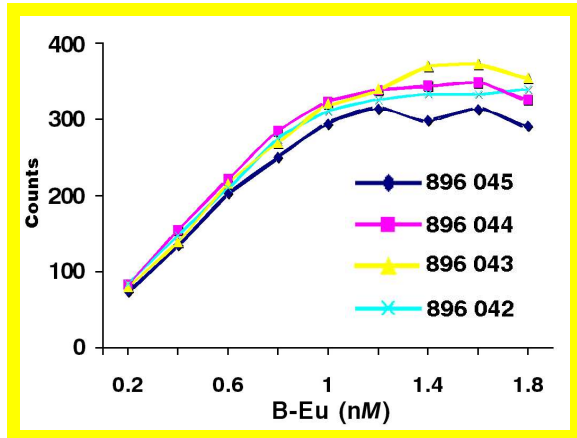


Figure 1 - Fluorescence at 665 nm for 0.2 nM SA-APC and various levels of B-Eu after 24 h.

The average deviation between different determinations within individual lots (4 - 23% for 1.8 nM B-Eu at 18 h) was greater than the average deviation between the mean 665 nm signal for different lots of SA-APC (3%), indicating that differences in 665 signal observed between the different lots were within the experimental limits of measurement of the assay. Eu fluorescence (615 nm) and the ratio of APC fluorescence to Eu fluorescence (665 nm:615 nm) were also similar for all lots tested (Figures 2 and 3).

The maximum 665/615 ratios occurred at 0.8 nM for all the conjugates tested (after more than 1 h incubation). This is consistent with full binding of SA's tetrameric binding sites, based on 0.2 nM SA in the assay.

Ten minutes were insufficient to achieve equilibrium at these low concentrations (Figures 4, 5 and 6). Incubation times longer than 1 hour were required to achieve the maximum 665 signals at the lowest concentrations (*i.e.* <0.8 nM B-Eu) tested.

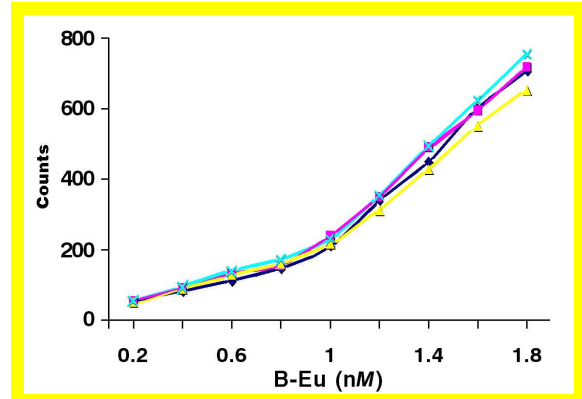


Figure 2 - As in Figure 1, fluorescence at 615 nm.

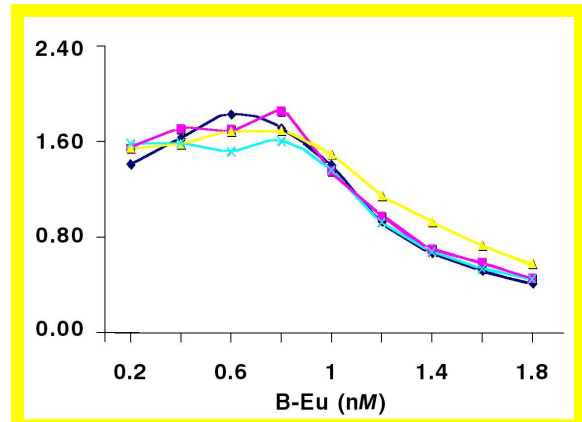


Figure 3 - As in Figure 1, ratio of fluorescence at 665 nm to fluorescence at 615 nm.

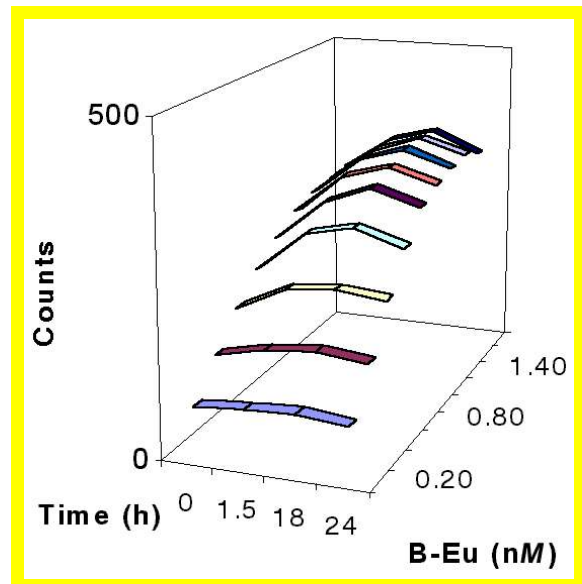


Figure 4 - Time dependence of 665 nm fluorescence, average of all determinations.

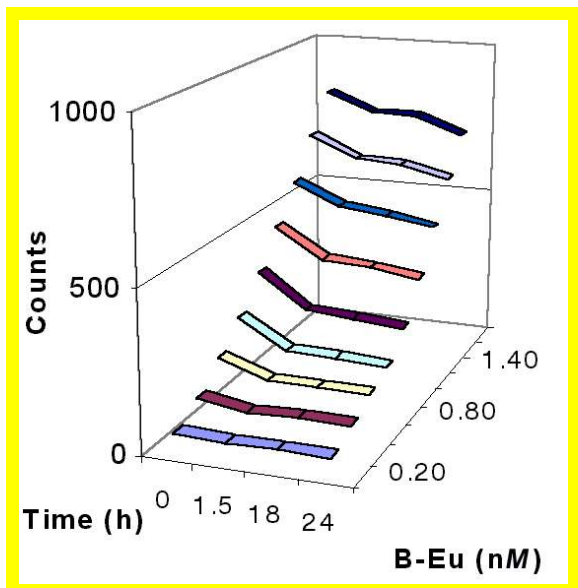


Figure 5 - As in Figure 4, fluorescence at 615 nm.

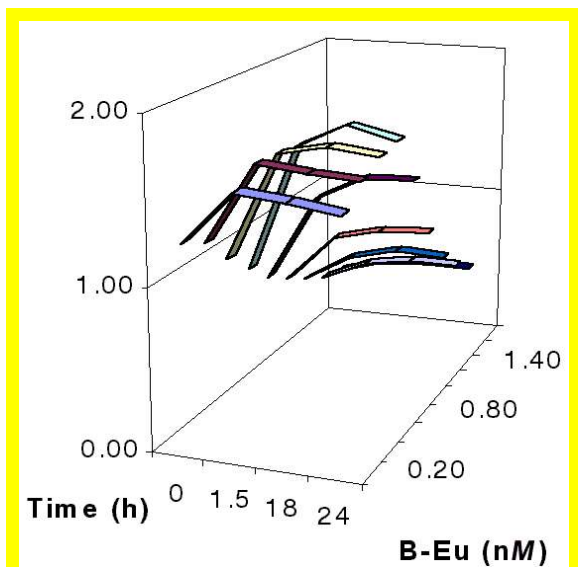


Figure 6 - As in Figure 4, ratio of fluorescence at 665 nm to fluorescence at 615 nm.

We observed no significant differences in the results when the plates were stored at room temperature *vs.* 4°C during the course of the experiment (24 h). This is consistent with long-term storage data demonstrating the excellent stability characteristics of PJ25S conjugates with regard to biotin-binding and APC fluorescence.

DISCUSSION

The results obtained demonstrate ProZyme's ability to provide multiple lots of SA- APC with highly consistent performance characteristics. ProZyme's control of all stages of the manufacturing process, including the manufacture of SA and APC components of the final product, is reflected in highly consistent performance.

The quantitative 4:1 binding of B-Eu and SA-APC was not unexpected due to the small size of the molecules involved. However, in the case of Eu-labelled macromolecules, steric hindrance may reduce the amount of Eu required to achieve saturation and may also reduce the maximum 665 signal. ProZyme is currently working to develop a performance assay with macromolecular Eu conjugates that will provide information supplementary to that presented here.

The performance tests reported here continue to be performed with both older lots of SA-APC and new production lots as they are completed. Such performance testing, combined with the test for macromolecular binding currently in development, will provide the basis for performance-based product certification.

TECHNICAL SERVICE

This and other TechNotes are available on PROZYME's webpage located at:

<http://www.prozyme.com/technical/index.html#technotes>

PROZYME customers are an important source of information regarding advanced or specialized uses of our products. We encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Victor and Lance are trademarks of PerkinElmer Life Sciences, Boston, MA, USA.

ProZyme and Phycolink are registered trademarks of ProZyme, Inc, San Leandro, CA, USA.



1933 Davis Street, Suite 207
San Leandro, CA 94577-1258

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

E-MAIL info@prozyme.com
WEB www.prozyme.com