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Room Temperature Stability Study of Streptavidin-Phycoerythrin Reporter Conjugates Using a Model TSH Immunoassay

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Abstract

Luminex® xMAP® technology has proven to be suitable for a wide variety of applications. Increasingly, this includes field applications where storage conditions are not as ideal as in laboratory settings. Reagent stability is always a concern for assay manufacturers, but in these cases there is a special need to demonstrate reagent stability. Utilizing a well-characterized sandwich immunoassay for human thyroid stimulating hormone (TSH), we demonstrate that SA-PEs designed specifically for the Luminex platform can be stored at room temperature for extended periods with no loss of performance.

Introduction

ProZyme offers fluorescent conjugates for many applications under its PhycoLink® product line. Over the years, ProZyme has refined a selection of Streptavidin-Phycoerythrin reporter conjugates (SA-PEs) specifically optimized for xMAP applications. Three different types of SA-PE (each manufactured by a different process and denoted by a different product code) have proven to be especially suitable:

- PJ31S ProZyme's original high-signal SA-PE; longest manufacturing history.
- PJRS20 Newer SA-PE; our highest-signal conjugate.
- PJRS34 Another new SA-PE, designed to minimize non-specific background problems sometimes encountered with certain antibodies.

In the present study we utilize a previously described and well-characterized TSH sandwich immunoassay (Baker, HN 2002; Amsden, B *et al.* 2009) as a model assay to evaluate the stability of different SA-PE samples stored at room temperature for extended periods. We will show that the tested SA-PEs are highly stable at room temperature. This finding is consistent with historical feedback from users attesting to the stability of these SA-PEs in xMAP assays and other applications.

Methods

Coupling of capture antibody to microspheres. Anti-TSH monoclonal antibody (clone 057-11003 from OEM Concepts, catalog number MAT04-003) was covalently coupled to polystyrene xMAP carboxylated microspheres (Microplex™ Microspheres) using a procedure recommended by Luminex Corporation (Sample Protocols for Immunoassay using Luminex Microspheres, pp. 10-11, Luminex Corporation, April 2007).

Biotinylated detection antibody. The detection antibody (Medix Biochemica clone 5403, obtained from BiosPacific) was biotinylated using EZ-Link®-Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific).

TSH stock solutions. Human thyroid stimulating hormone (TSH) was obtained from Scripps Laboratories (T0113). Stock solutions were prepared in PBS + 4% BSA, aliquotted to minimize multiple freeze-thaw cycles and were stored frozen.

Evaluation of SA-PE using a TSH standard curve. Signal strength of each SA-PE was evaluated by preparing 12-point standard curves of TSH starting at 1000 µIU/ml and serially diluting in half-log (factor of 3.16) increments.

1. Antibody-coupled microspheres were diluted to 10^5/ml in PBS-TBN; 20 µl were mixed with 20 µl of diluted TSH in a 96-well microplate. The plate was incubated for 60 minutes in the dark at room temperature with shaking at 500 - 600 rpm.
2. Biotinylated detection antibody was diluted to 4 µg/ml in PBS-TBN; 20 µl were added to each sample and the plate was incubated for 30 minutes in the dark at room temperature with shaking at 500 - 600 rpm.
3. SA-PE was diluted to a standard concentration (20 µg/ml) in PBS-TBN; 20 µl were added to each sample and the plate was incubated for 15 minutes in the dark at room temperature with shaking at 500 - 600 rpm.
4. Assay reagents were removed by vacuum filtration in pre-wetted wells of 96-well filter microplates (Multiscreen®$_{HTS}$ from Millipore Corporation), then the microspheres were washed twice with 200 µl of PBS-TBN. The microspheres were resuspended in 100 µl of PBS-TBN and read at high calibration on a Bio-Plex™ 200 instrument running the Bio-Plex Manager™ Software v5.0. Each test was performed in quadruplicate and averaged.

Treatment of outlying data points. In some cases, 3 of the 4 quadruplicate measurements were tightly grouped with the fourth point differing substantially from the other three. In such cases, the fourth point was eliminated from the calculated average if it was more than four times the standard deviation from the mean of the other three points.

Stability testing of SA-PEs. SA-PEs were tested in their standard bulk formulation (2 mg/ml in 10 m*M* Tris-HCl, 150 m*M* NaCl, 1 µg/ml pentachlorophenol, pH 8.2). 500-µl samples of each SA-PE were dispensed to polypropylene microcentrifuge tubes and were stored at room temperature (which ranged between 20 and 23°C) in the dark.

At intervals as indicated in the Results section, each SA-PE was assayed in quadruplicate as described, as was the parent lot (stored at the standard temperature of 4°C) from which the test sample was dispensed. The test sample and parent lot were assayed at the same time on the same plate using the same reagents.

Figure 1 Log-log plot of fluorescence intensity *vs.* human TSH concentration using PJRS20 SA-PE stored at 4°C and at room temperature

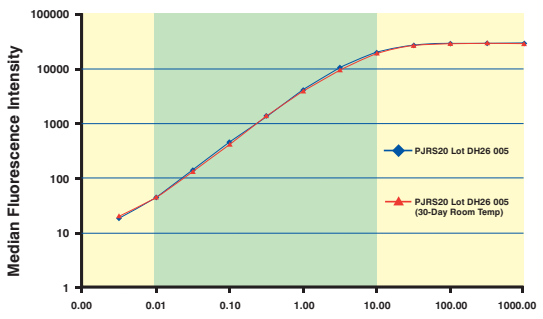
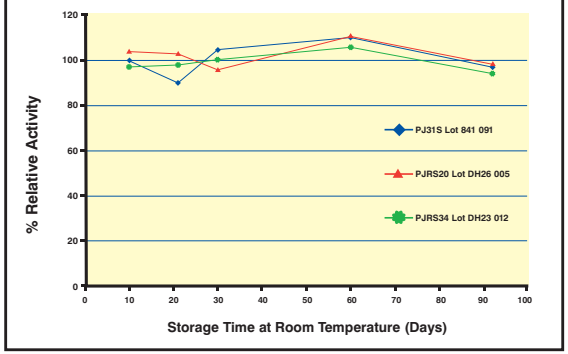


Table 1 Calculation of average relative activity of room temperature SA-PE compared with standard storage (4°C)

TSH (µIU/ml)	Parent Lot	Stability Sample	% of Parent Lot
1000	30208.8	28975.3	95.9
316	29924	29942.1	100.1
100	29105.5	29258	100.5
32	27309	27193.3	99.6
10	19944.4	19554.4	98.0
3.2	10599.4	9598.5	90.6
1.0	4158.5	3958.4	95.2
0.32	1345.1	1394.4	103.7
0.10	461.6	419.4	90.9
0.032	141	131.6	93.3
0.010	45.4	45.1	99.3
0.003	18.6	20.1	108.1
Average % of Parent Lot (.01 – 10 µIU/ml)			**95.9**

Figure 2 Stability of SA-PE stored at room temperature compared with standard storage (4°C)



Results and Discussion

Three different types of SA-PE (each designed for high performance in xMAP applications) were tested for stability as described in Methods. For each of these SA-PEs, a sample of a representative lot was stored in the dark at room temperature as described in Methods. Periodically, these samples were tested side-by-side with samples from the bulk supply of the same lot, which was kept at the standard storage temperature.

A model xMAP sandwich immunoassay for measuring TSH was constructed as described in Methods. This assay was used to measure performance of the SA-PE samples in this study. The samples were tested using a 12-point TSH standard curve, using TSH concentrations from 0.0032 - 1,000 µIU/ml, with quadruplicate measurements averaged and graphed as a function of the TSH concentration.

Figure 1 shows a representative comparison. In this case, a sample of SA-PE PJRS20, lot DH26 005 (the "parent lot") was tested side-by-side with a sample of the same material stored at room temperature for 30 days ("the sample"), as described previously. Figure 1 is typical of the results generated in this study, in that the TSH assay curve generated using the sample is closely similar to that of the parent lot throughout the range of TSH concentrations used in the test.

A proper comparison of parent lot and sample requires that the comparison be limited to the range of TSH concentrations in which the signal is proportional to the TSH concentration. The highest TSH concentrations in the curve produce MFI signals which are at the maximum level which can be recorded, and the lowest TSH concentration is near enough to background levels, that these points do not allow detection of a performance difference if it were to exist.

Thus, in Figure 1 the region in which the signal is dependent on the TSH concentration is shaded. Results in this region will be used to compare the performance of the parent lot to that of the sample.

Table 1 shows how this comparison was done for each test. This table shows the values that were plotted in Figure 1. At each TSH concentration, the average MFI (average of quadruplicate measurements) of the sample is expressed as a percentage of the same value for the parent lot. The shaded values in Table 1 correspond to the shaded region of the curve in Figure 1. The shaded percentage values were themselves averaged to generate the value recorded as "Average % of Parent Lot".

Figure 2 shows stability plots generated from measurements taken after storage at room temperature for different lengths of time. Data were collected as shown in Figure 1 and were used to calculate percent activities compared to the appropriate parent lots, as shown in Table 1.

Figure 2 shows that the activity of the stability samples relative to their respective parent lots is ±11% throughout the time period of the study (92 days) for each of the SA-PEs tested.

Conclusions

- The TSH assay described here is a useful assay for evaluating the performance of SA-PE in sandwich immunoassays.
- The long-term stability of these SA-PEs at room temperature is excellent. The samples show no significant loss of performance relative to their parent lots throughout the 92-day length of the study.
- Extended stability at room temperature is predictive of still longer stability at the standard storage temperature (2-8°C). Furthermore, it is consistent with reports from users of excellent long-term stability of these SA-PEs in a variety of applications including xMAP technology.
- Extended stability at room temperature also demonstrates the robustness of these SA-PEs to temperature excursions such as may occur during shipping or power outages, or in work environments where refrigeration is imperfect or absent.
- ProZyme will utilize this and other xMAP assays to continue to ensure high quality, demonstrate long-term stability and provide continuous improvement of its line of SA-PE reporter conjugates.

References

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