Dye Photobleaching

In all KinExA® experiments a fluorescent molecule is used to give a signal that can be interpreted by the instrument. Fluorescent molecules will lose activity when exposed to light. This loss in activity, called photobleaching, is directly proportional to the amount of light exposed to the label. It is therefore good practice to limit extraneous light for any sample that contains a fluorescent dye. Many of our scientists and customers routinely wrap their label solutions in foil to minimize photobleaching from extraneous light.

We had never bothered to quantify the photobleaching risk from extraneous light since using foil had no downside. Recently, with the autosampler, there have been incidents of a label tube being out of position due to the foil wrapper. It therefore made sense to try to quantify the amount of photobleaching that could be expected to occur if the protective foil was not used. Our finding is that photobleaching is insignificant in typical KinExA experiments.

Details

We wanted to have our test conditions simulate the typical experimental environment, so we started with the most popular dye used in KinExA experiments, Cy5. Recommended running concentration of this label is 1 µg/mL, so this was the concentration used in the stability experiments. A test sample of the label was made, then split into two identical vials. One vial was wrapped in foil and the other was left exposed to room light. The samples were tested periodically for fluorescence intensity.

In an initial experiment there was no detectable decrease in fluoresence level in either sample after 4.5 hours, the time of a typical KinExA experiment. Another test was run over a substantially longer time to allow enough change in the exposed sample to get an accurate measure of the rate of photobleaching in unprotected samples. The trial was run for 4 days, with the room light being left on 24 hours per day.

A photobleaching decay term in percent per hour was fit to the data. In the 4 day Cy5 experiment (***Figure 1***) decay was 0.01% per hour for the exposed sample, and 0.02% per hour for the foil wrapped sample. Even after 4 days of continuous light exposure there was not a measurable drift.

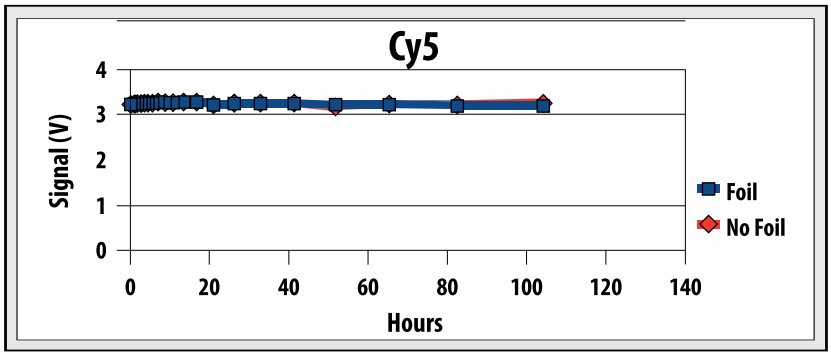

Figure 1: *The decrease in signal over time for Cy5 under continuous lab light over the course of 105 hrs. Blue data points represent replicate samples with foil and the red data points represent replicate samples without foil.*

Since we still could not see any photobleaching, we tried an "extreme" lighting experiment, where the exposed sample was placed next to a fluorescent light bulb. We estimated the light level to be at least 10 times higher than previous conditions. With extreme lighting we were finally able to measure photobleaching of the Cy5 dye, although it was only 0.6% per hour. This level of drift would be barely noticeable in a normal experiment and was done with a lighting level that would never be encountered in a real experimental setup.

Cy5 is a very photostable dye, so we then used one of the least photostable dyes: FITC. The FITC experiment was run for 6 days, again with lighting left on 24 hours a day (***Figure 2***). Unlike Cy5 dye, we were able to measure a photobleaching drift under normal room light conditions, but on a time scale much longer than experiments.

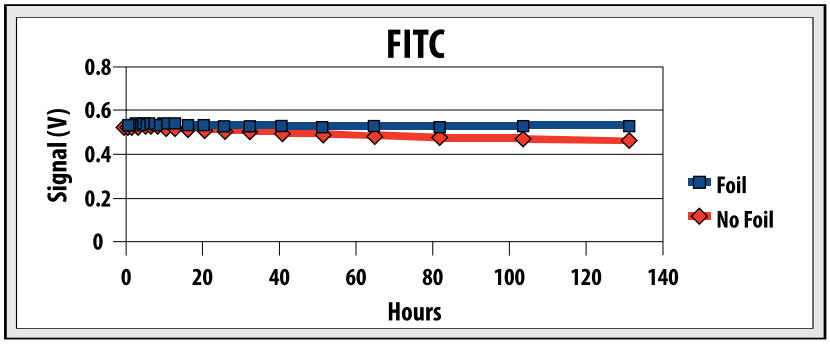

Figure 2: *The decrease in signal over time for FITC under continuous lab light. The blue data points represent replicate samples with foil and the red data points represent replicate samples without foil.*

Analysis of the FITC data showed a drift of 0.11% per hour. This level of drift over a 4.5 hour experiment would only be a total drift of 0.5%, which would quite likely be less than the other noise sources in the experiment.

Conclusion

Results demonstrate that photobleaching is insignificant under normal lab lighting and to unprotected samples. Based on these findings, use of foil to prevent photobleaching is not recommended as it does not improve results, and can cause unintended failures in experiments. There have been several cases where foil used on label tubes displaced the tube enough to cause flow problems on the autosampler. For customers who wish to still use foil, we do recommend that the entire conical section at the bottom of the tubes be left free of foil.