High Dynamic Range (HDR) Immunoassay for the Multiple Simultaneous Quantification of Cytokines

Chris Lyman, Abby Tyler, & Sara Wallace
Quansys Biosciences
Logan, Utah

Abstract

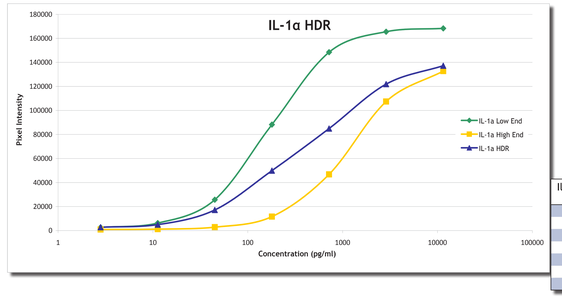
Traditional ELISAs are unable to achieve high sensitivity while maintaining the ability to quantify large analyte concentrations. Faced with these limitations, researchers have had to use more time, sample, and money to test extra dilutions of their samples. However, the new Q-Plex™ High Dynamic Range (HDR) developed by Quansys Biosciences greatly extends the quantifiable range for any given assay, producing calibration curves that span a 3-5 log range. These extended and more sensitive ranges allow researchers to more efficiently quantify cytokines without the need for multiple sample dilutions, greatly improving throughput and reducing the cost of running the assays. The power of the Q-Plex™ HDR platform was demonstrated for IL-1α, IL-4, IL-6, and TNFα.

Introduction

Immunoassays measure the concentration of an analyte in a liquid using the specific reactions of antibodies to their antigens. The innovation of multiplexing facilitates simultaneous measurement of multiple analytes in a high-throughput format. This does not, however, address the inability of immunoassays to synchronously quantify at both very high and low concentrations. To avoid the taxing multiplicity of operations that is often employed to detect analyte concentrations at their extremes, the quantifiable range must be extended.

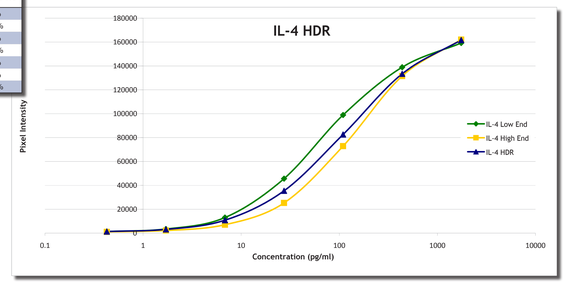
In an effort to overcome the problem of limited quantifiable range, Quansys Biosciences has developed a planar-based assay platform that enables measurement of highly concentrated analytes and extends low-end sensitivity. This is accomplished by “multiplexing” two assays for each analyte in an array, each optimized to measure a specific region of the entire theoretical range of the reagents used. Not only does the technology extend the quantifiable range, it is also suitable for multiplexing and compatible with high-throughput systems. The new platform is called the High Dynamic Range (HDR) Q-Plex™ Array.

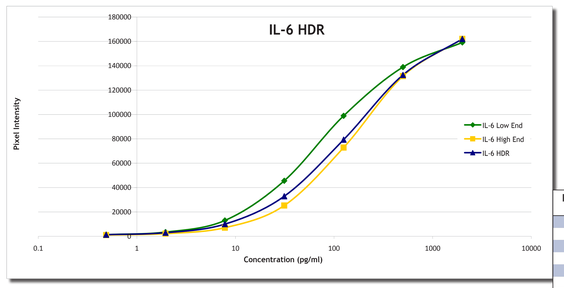
These methodologies have been applied to the development of a 4-plex cytokine array: IL-1α, IL4, Il-6, and TNFα. Within this array, each target antigen is measured by two distinct assays. The result is an extended quantifiable range for each of the four analytes tested.



Il-1α Standard (pg/ml)	Il-1α HDR Back-fit (pg/ml)	% Back-fit
11500	11505.8	100%
2875	2875.3	100%
718.8	718.0	100%
179.7	180.2	100%
44.9	44.5	99%
11.2	12.1	108%
2.8	1.9	67%

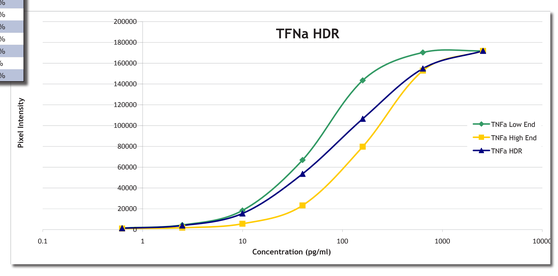
Il-4 Standard (pg/ml)	Il-4 HDR Back-fit (pg/ml)	% Back-fit
1750	1551.7	89%
437.5	439.3	100%
109.4	108.2	99%
27.3	27.7	101%
6.8	6.7	98%
1.7	1.7	99%
0.4	0.5	117%





Il-6 Standard (pg/ml)	Il-6 HDR Back-fit (pg/ml)	% Back-fit
2000	2003.5	100%
500	493.3	99%
125	126.3	101%
31.3	30.7	98%
7.8	8.2	105%
2.0	1.8	91%
0.5	0.5	98%

TNFα Standard (pg/ml)	TNFα HDR Back-fit (pg/ml)	% Back-fit
2550	2548.0	100%
637.5	637.8	100%
159.4	159.3	100%
39.8	39.9	100%
10.0	10.0	100%
2.5	2.4	97%
0.6	0.7	114%



Materials and Methods

Matched antibody pairs were obtained from commercial sources. Capture antibodies were printed onto 96-well plates at Quansys Biosciences. Each capture antibody was deposited twice within each well: the first in a manner that would ensure maximum low-end sensitivity and the second in a manner optimized for high-end quantification. Assay conditions were optimized using standard ELISA methodologies

Human PBMCs were harvested and grown using cell culture techniques. Cell supernatants were obtained, then Il-1α, Il-4, IL-6, and TNFα levels were measured using the Quansys HDR Q-Plex™ platform. The resulting chemiluminescent signal was recorded using the Quansys Q-View™ digital imaging system.

A five-parameter logistic model was fit to the standard data for each spot, resulting in two standard curves for each system. Maximum-likelihood estimation was then used to solve for concentrations based on both curves but weighted toward the data with the best precision profile and goodness-of-fit.

Conclusion

The HDR Q-plex™ platform was able to accurately quantify multiple cytokines simultaneously over a significantly broader range than traditional ELISAs. In fact, the HDR platform covered two orders of magnitude greater than many other ELISA kits available on the market. In our HDR Q-Plex™ experiment, this greater dynamic range was advantageous when measuring IL-1α, Il-4, Il-6, and TNFα in the PBMC supernatants in multiple ways. Not only did the extended range make simultaneous quantification over many logs easier preparation-wise, it also increased sensitivity without sacrificing high-end measurements. Specifically, we were able to simultaneously, accurately measure IL-4 at low concentrations and report IL-6 and TNFα at relatively high concentrations at the same time. The HDR Q-Plex™ platform enables users to save even more time, sample, and money by facilitating the measurement of multiple cytokine levels from sub-picogram to high nanogram per milliliter levels using fewer sample dilutions.