A STELLUX™ Chemiluminescence ELISA for Broad Range and Sensitive Detection of Human Insulin

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Abstract

Quantification of insulin in serum or plasma over a wide range of disease states or pre and post drug treatment is vital in furthering diabetes research. Many commercial human insulin ELISAs require extra sample dilution steps to ensure concentrations fall within a limited standard curve range. This results in increased sample preparation time, as well as potential re-dilution and re-testing of samples. ALPCO has developed a broad range, highly sensitive, and small volume human insulin ELISA to address these issues.

Introduction

Insulin is a peptide hormone characterized in over 100 vertebrate species[1] and plays an important role in the regulation of vertebrate metabolism.[2] The number of people with type 1 diabetes (T1D), characterized by insufficient insulin secretion due to a deficit in pancreatic beta-cell mass, and type 2 diabetes (T2D), characterized by impaired glucose-mediated insulin secretion and insulin resistance, is estimated to be over 360 million by the year 2030.[3,4,5] Undoubtedly, insulin is a widely studied biomarker for furthering diabetes research. In addition, insulin plays an important role in tissues of the brain and the vascular endothelium. It mediates the serine/threonine kinase (AKT), also known as protein kinase B (PKB), pathway that leads to the activation of endothelial nitric oxide synthase (eNOS) and increased nitric oxide (NO) production in the vascular endothelium.[6] Insulin dysfunction is also involved in neurological disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and mental disorders like depression and psychosis.[7]

For over 40 years, enzyme-linked immunosorbent assays (ELISAs) have been the primary method for detecting analyte concentrations in aqueous solutions.[8] There are several variations of ELISAs with 88% of the market using the traditional horseradish peroxidase (HRP)-colorimetric detection chemistry, followed by the alkaline phosphatase (AP)-colorimetric method and then the HRP-chemiluminescent detection system.[8] Primary motivating factors for adopting a new ELISA platform, like the STELLUX™ chemiluminescence system, are improved assay sensitivity, better assay uniformity, increased assay speed, decreased assay time, and thus higher throughput.[8]

Insulin secretion and sensitivity are important clinical characteristics to monitor when evaluating the long term success of insulin independence in patients having undergone islet transplantation.[9,10] Consumer preference for high assay sensitivity in conjunction with routine monitoring of insulin levels during transplant studies illustrates the importance of offering a high throughput and broad range immunoassay for measuring insulin levels.

Critical Performance Attributes

Until recently, an immunoassay that could accurately measure a wide range of insulin concentrations from 5-50,000 pg/mL in human samples was not commercially available. Furthermore, a 2012 HTStec's ELISA Assay Trends report indicated an increase in assay sensitivity and reduction of sample consumption as being the two highest ranking factors of consumer importance when improving ELISA performance.[8] Factors which ALPCO took into careful consideration while developing a new chemiluminescent method for analyte detection.

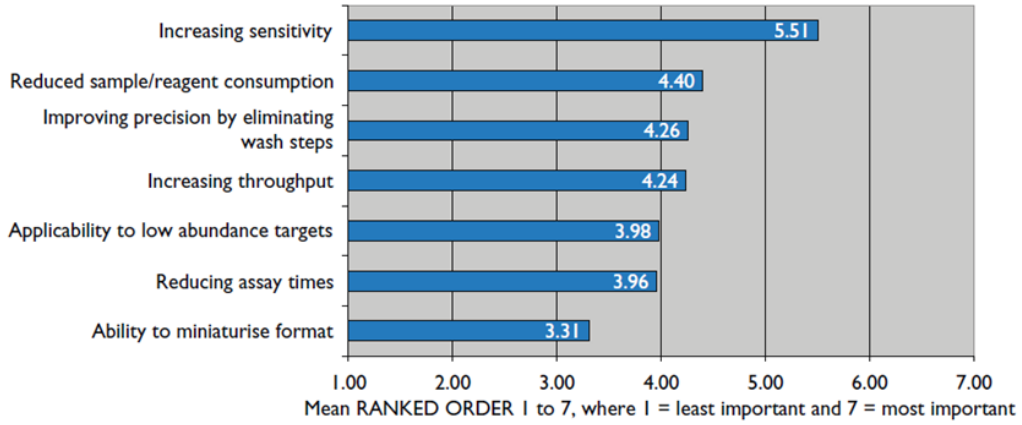

Figure 1: *Rankings for the Leading Issues with ELISA Performance. Copyright HTStec 2012.*[8]

STELLUX™ Solution

The STELLUX™ Chemiluminescent Human Insulin ELISA offers an optimal solution for accurate, sensitive and wide range detection of insulin using as little as 25 µL of sample. The broad dynamic range significantly decreases sample preparation time by eliminating dilution steps and takes the guesswork out of determining ideal dilution factors, while the low sample size of the assay ensures reduced sample consumption. These features aid in preserving the customer's precious samples and helps labs save time and money with higher throughput. Moreover, the increased sensitivity enables consumers to accurately measure a variety of samples ranging from normal, to those with impaired glucose tolerance and those with type 1 or type 2 diabetes.

The Chemi Human Insulin assay (80-INSHU-CH01) is currently for Research Use Only and was developed for the quantitative determination of insulin in human serum, plasma, and tissue culture samples over a dynamic range of 5-30,000 pg/mL with results achieved in 1 hour and 35 minutes. Analytical (LOD) and functional sensitivities are 1.51 pg/mL and

STELLUX™ Solution *Continued*

10 pg/mL respectively. Below are key features of the assay and how they compare amongst other kits on the market:

	ALPCO STELLUX™	Millipore	Mercodia	Life Technologies	Meso Scale Discovery
Assay Type	Chemiluminescent ELISA	HRP-Colorimetric ELISA	HRP-Colorimetric ELISA	HRP-Colorimetric ELISA	Multi-Array
Sample Size	25 µL	20 µL	25 µL	50 µL	25 µL
Sample Type	Plasma, Serum, Tissue Culture	Plasma, Serum, Cell Culture	Plasma, Serum	Plasma, Serum	Plasma, Serum
Range	5 - 30,000 pg/mL	69.6 - 6,960 pg/mL	104.4 - 6,690 pg/mL	174 - 17,400 pg/mL	3.2 - 50,000 pg/mL
Analytical Sensitivity (LOD)	1.51 pg/mL	34.8 pg/mL	34.8 pg/mL	5.92 pg/mL	7.5 pg/mL
Functional Sensitivity	10 pg /mL	Not Available	Not Available	Not Available	Not Available
LLOQ	20 pg/mL	Not Available	Not Available	Not Available	Not Available
ULOQ	24,000 pg/mL	Not Available	Not Available	Not Available	Not Available

Figure 2: *Benchmark characteristics of various human insulin assays on the market*

Sample correlation was evaluated at two independent laboratories and results generated from this study were compared to results generated in-house at ALPCO. As seen in Figure 3, there is a high degree of inter-laboratory correlation with an r^2 value of 0.999.

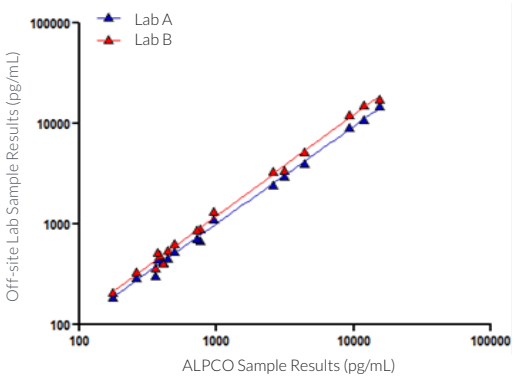

Figure 3: *Inter-laboratory correlation data*

Additionally, correlation between the ALPCO human insulin colorimetric ELISAs and the STELLUX™ chemiluminescent platform was evaluated. Results showed the ability to quantitate samples similarly on both kits, ensuring customers will be able to smoothly transition from the colorimetric method to the chemiluminescence platform.

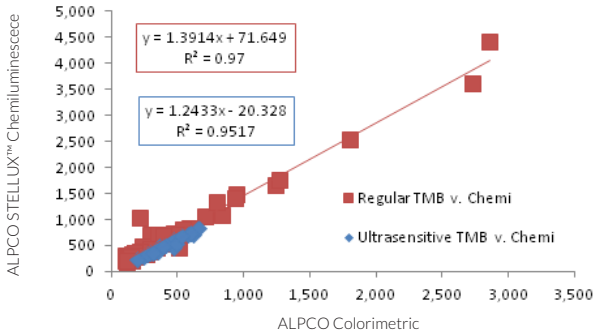

Figure 4: *Correlation of ALPCO's STELLUX™ Human Insulin assay to ALPCO's Regular and Ultrasensitive Human Insulin assays*

Benefit of Chemiluminescence

Unlike other test platforms, ALPCO's line of chemiluminescent assays does not require expensive high-end instrumentation. The STELLUX™ ELISAs are 96-well black plate assays utilizing a standard chemiluminescent (glow) reader common in most laboratories. Aside from the increased sensitivity and broad dynamic range, the chemiluminscent platform also allows for analyte detection with a variety of different sample types including serum, heparin plasma, and tissue culture supernatants with minimal sample preparation required. These features make the STELLUX™ chemiluminescent kits ideal for use in a range of testing facilities including contract research organizations, core reference laboratories, academic institutions and pharmaceutical companies performing pre-clinical research.

Moreover, multiple lots are validated against each other for parameters that include functional sensitivity, inter and intra assay precision, accuracy, LLOQ, ULOQ, and linearity of dilution. This gives consumers reassurance on the robustness of the assay. Plus, the high correlation to ALPCO's colorimetric insulin ELISAs makes for an easier transition to the new STELLUX™ chemiluminescent platform for existing users, while the inter-laboratory correlation builds confidence in the accuracy of results.

Summary

ALPCO's STELLUX™ Chemiluminescent Human Insulin ELISA offers broad range detection with just a 25 µL sample volume, significantly reducing sample preparation time by eliminating dilution steps and the guesswork involved in determining ideal dilution factors. It also provides the best sensitivity on the market with quantitative detection of insulin as low as 10 pg/mL and no cross-reactivity to mouse/rat insulin or human intact proinsulin, ensuring results from pre-diabetic or Type I diabetic samples are accurate and dependable.

For more information on the STELLUX™ assays please contact us at:

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Email: Russ Jarres at rjarres@alpco.com

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