A Broad Range, Highly Sensitive, Small Sample Volume Chemiluminescent ELISA for Measuring Insulin in Human Samples

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STELLUX™ chemiluminescence

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ABSTRACT

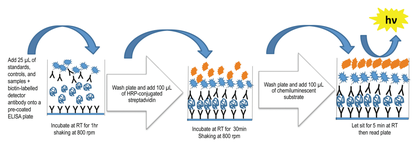
Quantification of insulin in serum or plasma over a wide range of disease states or pre and post drug treatment is vital in furthering diabetes research. Many commercial human insulin ELISAs require extra sample dilution steps to ensure concentrations fall within a limited standard curve range. This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

The STELLUX™ Human Insulin Chemiluminescent ELISA has been 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. Analytical and functional sensitivities are 1.51 pg/mL and 10 pg/mL, respectively. Sample linearity for human samples (serum and heparin plasma) ranges from 84-114% with r^2 values of 0.997. Samples spiked with 3 levels of insulin recover at averages of 90%, 92% and 92% at the high, mid, and low spikes, respectively. Serum and plasma concentrations from normal, fasted or fed diabetic and non-diabetic human samples range from 81 pg/mL to 6,627 pg/mL (n=42). Inter- and intra- assay precision are at <20% for n=48 over two days.

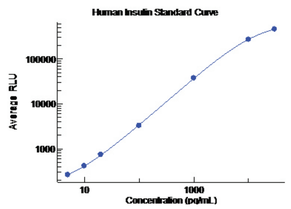
ALPCO has developed a highly sensitive and broad-ranged chemiluminescent assay that uses 25 µL of sample and achieves results in 1 hour and 35 minutes. The assay also eliminates the need for sample dilution, reducing assay time and reagent cost, leading to greater potential savings for high volume screening labs.

MATERIALS AND METHODS

The STELLUX™ Human Insulin Chemiluminescent ELISA is a sandwich assay, which uses two mouse monoclonal antibodies: one as a capture antibody that is adsorbed to a 96 black-well microplate and the other as a primary detector antibody conjugated to biotin. Standards and/or samples and controls are added to the plate followed by the addition of the biotinylated conjugate. The antibody-antigen-antibody sandwich is then incubated at room temperature on a plate shaker set at 800 rpm. Post incubation, the plate is washed six times, horseradish peroxidase (HRP)-conjugated streptavidin is added to each well, and incubated at room temperature on a plate shaker set at 800 rpm. Post incubation, the plate is washed once and the chemiluminescent substrate, LumiGLO®, is added to each well. The microplate is read on an appropriate plate reader (sensitivity of 150 for 1 second) 5 minutes post substrate addition. The resulting relative light units (RLUs) are directly proportional to the amount of insulin present in the sample.



STANDARD CURVE



Concentration (pg/mL)	RLU Average	CV RLU (%)	S/N ratio
30,000	521,996	15.2	3457
10,000	307,351	10.7	2035
1,000	40,391	6.3	267
100	3,669	8.5	24.3
20.0	862	12.6	5.71
10.0	474	12.5	3.14
5.00	332	22.4	2.20
0	151	31.0	

SENSITIVITY

n=36	RLU Avg	St. Dev	%CV	RLU Avg + 2.5 St. Dev	LOD Conc (pg/mL)
Assay Blank	122	35.9	29.5	241	1.51

Expected Conc (pg/mL)	RLU Avg	RLU %CV	Calc Conc Avg (pg/mL)	Conc %CV	% Recovery Avg	
30,000	533708	7.9	26,500	28.0	88	
27,000	513386	7.2	23,500	20.0	87	
24,000	498551	6.6	21,700	13.4	90	ULOQ

Expected Conc (pg/mL)	RLU Avg	RLU %CV	Calc Conc Avg (pg/mL)	Conc %CV	% Recovery Avg	
40	1698	6.7	45.2	6.8	113	
20	892	9.4	23.1	10.1	115	
10	430	14.1	9.88	17.9	99	LLOQ Functional Sensitivity

INTRA-ASSAY PRECISION

	Sample 1	Sample 2	Sample 3
Avg Conc (pg/mL)	11.7	496	14, 245
CV (%)	17.9	6.6	12.8

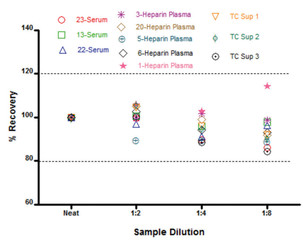
Determined from the mean and standard deviation (n=16) of three samples evaluated in a single assay

INTER-ASSAY PRECISION

	Sample 1	Sample 2	Sample 3
Avg Conc (pg/mL)	11.3	482	14,247
CV (%)	18.5	9.0	11.5

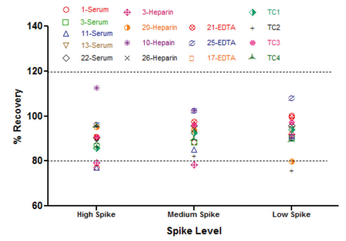
Determined from the mean and standard deviation (n=32) of three samples evaluated in 2 independent assays.

DILUTIONAL LINEARITY



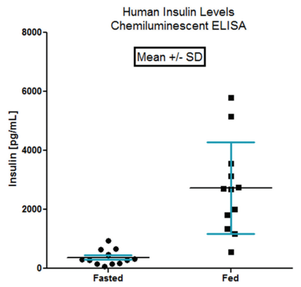
Serum, heparin plasma, and tissue culture were diluted in assay diluent to determine the linearity of dilution. The mean % recovery and r^2 value of all samples was 95% and 0.997, respectively.

SPIKE AND RECOVERY



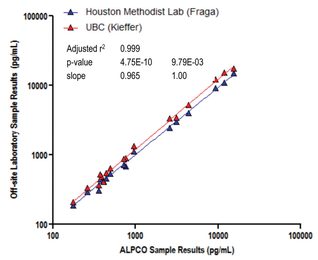
Three levels of insulin were spiked into human serum, heparin plasma, and EDTA plasma samples, as well as tissue culture supernatants to evaluate assay accuracy. The high, medium, and low spiked samples recovered at 90%, 92%, and 92%, respectively.

FASTED AND FED SAMPLES



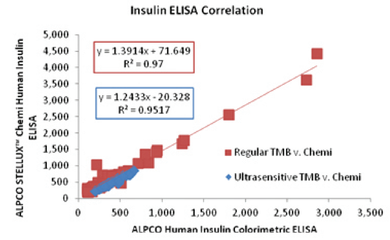
The assay was able to detect the difference between the fasted and fed sample groups

INTER-LABORATORY CORRELATION



17 Human samples were evaluated at two independent laboratories. The results from each of the two laboratories were compared to results generated at ALPCO and demonstrate a high degree of correlation.

ALPCO HUMAN INSULIN ELISA CORRELATION



STELLUX™ Human Chemiluminescent Insulin ELISA (80-INSHU-CH01, "Chemi") was evaluated with the current Insulin ELISA (80-INSHU-E01.1, "TMB Regular") and Ultrasensitive Insulin ELISA (80-INSHUU-E01.1, "TMB Ultra") to determine how all three assays correlate.

CONCLUSIONS

- A highly sensitive, reproducible, low volume, and broad-ranged assay
- Sensitivity: ≤2 pg/mL (LOD)
- Precision: <20% CV (inter and intra)
- Small sample size: 25 µL
- Broad dynamic range: 5 – 30,000 pg/mL
- Total Assay Time: 1 hour and 35 min
- Sample Types: Serum, Plasma, and Tissue Culture
- Inter-laboratory testing demonstrates a high degree of correlation (r^2 value>0.99)

ACKNOWLEDGMENTS

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