Application Note
Measurement of Glucose-stimulated Insulin Secretion from 3D InSight™ Islet Microtissues with ALPCO STELLUX® ELISA

High sensitivity and broad dynamic range of ALPCO STELLUX® Chemi Human Insulin ELISA enables reliable assessment of insulin secretion from highly standardized 3D InSight™ Islet Microtissue platform in a wide range of experimental windows.

Introduction and Background
Pancreatic beta cells (β cells) sense fluctuations in metabolic demand, particularly subtle alterations in circulating glucose levels, and respond in a coordinated fashion by secreting accurate amounts of insulin to maintain optimal glucose homeostasis and metabolism\(^1\). Loss of β-cell mass or impairment of normal insulin secretion results in diabetes, the most common metabolic disease in humans\(^1\). Due to the central role of pancreatic islets in the maintenance of normal glycaemia, anti-diabetic research and drug discovery is largely dependent on primary human islets as an in vitro model system. However, isolated islets present multiple limitations to their experimental use due to their inherent heterogeneity in size, cellular composition, function and purity\(^2,3,4\). Additionally, quickly after isolation they suffer from fading functionality (glucose responsiveness) and rapid decline in viability\(^5,6\).

Highlights
- High sensitivity of STELLUX® ELISA enables accurate quantification of basal insulin secretion from human islet microtissues
- A wide variety of experimental windows for glucose-stimulated insulin secretion from human islet microtissues is supported by the broad dynamic range of STELLUX® ELISA
Reliable assessment of insulin secretion in 3D InSight™ Islet Microtissues

To address these issues, InSphero developed a standardized islet model, 3D InSight® Islet Microtissues, produced by optimized dissociation and controlled scaffold-free reaggregation of primary islet cells. This patented process allows for precise control over the newly forming islet microtissue size and eliminates the contaminating exocrine material while ensuring homogeneous and native-like distribution of endocrine cells within each tissue. The resulting uniform islets, cultured in a one-islet per well format, display long-term (>28 days) and robust function enabling high-throughput and longitudinal study of pancreatic islet function, regeneration, and preservation. This miniaturized and robust pancreatic islet model, however, requires compatible end-point assessments, such as a sensitive enzyme-linked immunosorbent assay (ELISA) to measure the relatively low levels of basal insulin secreted during low glucose conditions. Additionally, InSphero recommends using an ELISA with a large dynamic range given that each islet can secrete up to 40 times higher amounts of insulin when stimulated with increasing concentrations of glucose. The STELLUX® ELISA technology provides several advantages that make it the preferred ELISA for this islet model: high sensitivity down to 5 pg/mL of insulin, broad dynamic range up to 30,000 pg/mL, and small sample size for preserving precious samples and enabling multiparametric end-point assessments. Here, we show how to use STELLUX® ELISA technology to reliably quantify levels of insulin, secreted under resting and induced conditions from standardized 3D InSight™ Human Islet Microtissues.

Materials and Methods

3D Microtissues, Media, and Assays

- 3D InSight™ Human Islet Microtissues in Akura™ 96 Plate (InSphero MT-04-002-01)
- 3D InSight™ Human Islet Maintenance Medium (InSphero CS-07-005-01)
- 2.8 and 16.7 mM D-Glucose solution in Krebs-Ringer HEPES Buffer (KRHB, InSphero CS-07-050-01) with 0.5 % bovine serum albumin (BSA, RIA grade, Sigma-Aldrich Cat. #A7888)
- STELLUX® Chemi Human Insulin ELISA, (InSphero and ALPCO 80-INSHU-CH01)

Glucose-stimulated Insulin Secretion

3D InSight™ Human Islet Microtissues were maintained in 3D InSight™ Human Islet Maintenance Medium. Culture media was removed, islet microtissues were washed twice in 70 µL KRHB with 2.8 mM glucose, then equilibrated for 1 hour in the same buffer solution. Glucose-stimulated insulin secretion (GSIS) was performed in 50 µL KRHB containing indicated glucose concentrations for 30, 60 or 120 min. The supernatant was collected for ELISA analysis. For a detailed protocol, please refer to InSphero Technical Protocol 013, “Assessment of β-cell function in 3D InSight™ Human Islet Microtissues.”

ALPCO STELLUX® Chemi Human Insulin ELISA

As per the kit instructions, standards, and supernatants were added together with the conjugate to the microplate, which was then incubated for 1 hour at room temperature on a plate shaker at 700 RPM. After washing, streptavidin-HRP conjugate was added to the wells for a 30 min incubation. The wells were then washed again followed by addition of the chemiluminescent substrate to each well. Quantification of the luminescent signal was performed using a Tecan Spark™ 10M Multimode Microplate Reader.

Results and Discussion

Insulin secretion from 3D InSight™ Islet Microtissues was evaluated using a glucose-stimulated insulin secretion test with static incubation in various glucose concentrations for 30, 60 or 120 minutes. Insulin concentrations in supernatants
were measured using a STELLUX® Chemi Human Insulin ELISA. A logarithmic standard curve was constructed, and sample concentrations were interpolated using the GraphPad Prism asymmetric sigmoidal 5-parameter curve-fitting function. Although curve fitting improves accuracy and precision of interpolation, all curve fitting models are limited at the lowest and highest ends of the detectable range where the analyte response becomes non-linear. Using the STELLUX® ELISA with 3D InSight™ Islet Microtissues, whether the GSIS assay was performed for 30, 60 or 120 min, basal (2.8 mM glucose), resting (5.5 mM glucose) and stimulated insulin secretions (16.7 mM glucose) were within the detection range of the assay (Figure 1).

3D InSight™ Islet Microtissues displayed a robust and glucose dependent insulin secretion with an average of 18-fold induction between basal and glucose-stimulated concentrations (Figure 2). As expected, total secreted insulin increased in a time dependent manner (Figure 2A). Insulin secretion per min, however, displayed very low variation among each individual experimental group with shorter incubation (30 min) resulting in a slightly superior rate of glucose-stimulated insulin secretion (Figure 2B). The more robust first phase of the biphasic insulin secretion (mediated by the limited readily releasable pool of insulin granules) starts about 5 min following glucose stimulation and lasts about 10 min, and thereby covers a bigger portion of the GSIS assay performed for 30 min. This phenomenon might partially explain the relatively higher secretion rates. Additionally, partial exhaustion of islet microtissues may occur after 2 hours of constant glucose stimulation. However, both due to the robust stimulation index (stimulated/basal insulin secretion) and small differences in secretion rates in all tested assay windows, we conclude that 3D InSight™ Islet Microtissue platform allows for assay time flexibility when performing glucose-stimulated insulin secretion if a compatible ELISA is used.

**Figure 1. Dynamic assay window allows quantification of basal and induced insulin secretion from islet microtissues.** Insulin was measured in 15 samples per time period (5 replicates per glucose concentration) using the STELLUX® Chemi Human Insulin ELISA. A logarithmic standard curve with sample values for 30, 60 and 120 min of insulin secretion is shown in relative luminescence units (RLU).
Figure 2. Reproducible insulin secretion independent of assay time. Insulin was measured in 25 samples per time period (5 replicates per glucose concentration) using the STELLUX® Chemi Human Insulin ELISA. A) Total insulin secretion and B) insulin secretion per minute in each sample is plotted. Insulin secretory function displayed by pg of insulin secreted per min showed a low variation between individual assay windows. Data represents mean ± SEM.

Conclusions
Given its high sensitivity and broad dynamic range, ALPCO STELLUX® Chemi Human Insulin ELISA enables reliable data acquisition when used in combination with highly standardized 3D InSight™ Islet Microtissue platform in a wide range of experimental windows. This combination of the two best-in-class systems, 3D InSight™ Islet Microtissue bundled with the ALPCO STELLUX® Chemi Human Insulin ELISA, is the method of choice for assessing insulin secretory function of human pancreatic islets in a reliable and accurate manner.

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References