The next level in insulin quantification assays

Guide to the next level in Insulin quantification assays

Discover our most Advanced Solutions for Insulin Quantification

Accurate quantification of insulin in various sample matrices is essential to preclinical studies of insulin secretion. Current assay strategies heavily rely on ELISA methods, involving multiple washing steps as well as a high price per point.

Revvity offers a comprehensive line of insulin quantification assays designed to significantly reduce the time to results, sample consumption, and cost of quantifying insulin. All Revvity assays have been extensively validated on relevant cellular models for insulin release and they show high correlation with established methods.

This brochure describes the functionality and benefits of all 3 assay kits available for insulin quantification.

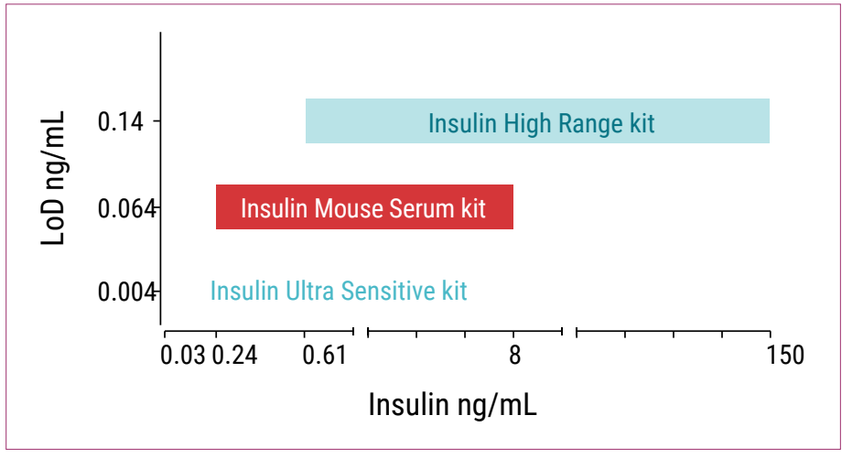


Fig 1: Three HTRF® assay options to choose from to quantify insulin

TABLE OF CONTENTS

HTRF Technology: Fast and Easy, High-Quality Quantification

Insulin is measured using a sandwich immunoassay of two monoclonal antibodies, one labeled with a donor fluorophore and one labeled with an acceptor fluorophore. The intensity of the FRET signal obtained is proportional to the concentration of insulin in the sample.

Designed for ease of use, the assay does not require lengthy extraction procedures or sample pre-treatment as may be the case for other technologies, nor does it require any washing steps. Simply add the detection reagents and read.

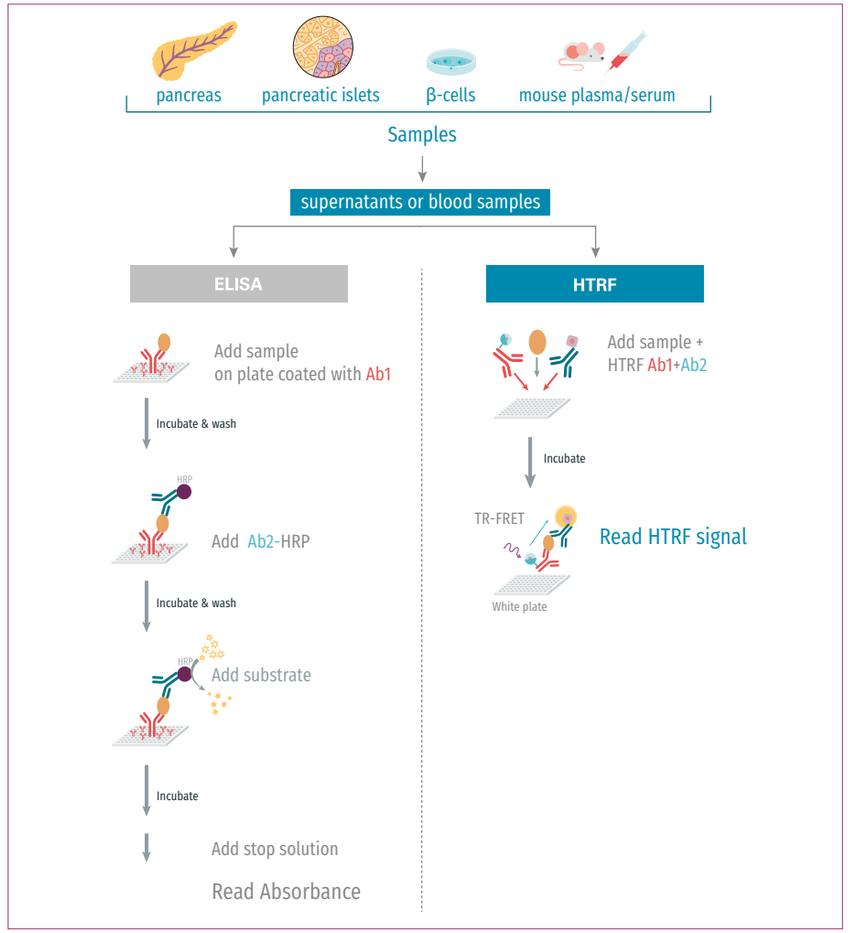


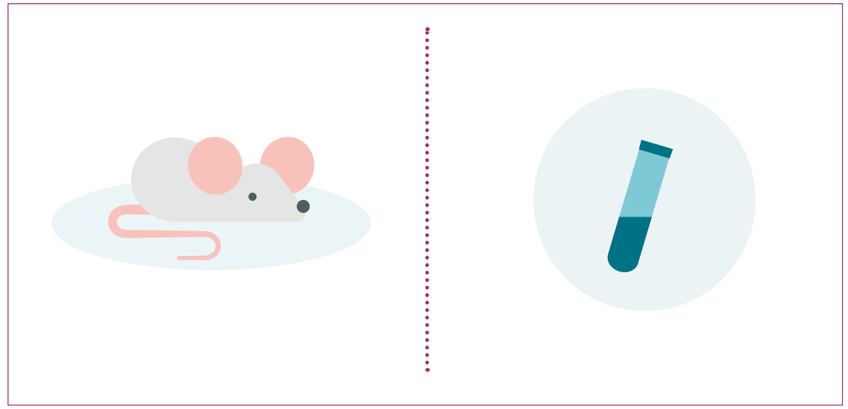
TABLE OF CONTENTS

Insulin Mouse Serum Assay Kit

Fast, accurate quantification of insulin in extremely low sample volumes

For years researchers have sought the ideal insulin assay which would combine absolute specificity with sensitivity, accuracy, low sample consumption, and ease of use. The search has come to an end.

Revvity's Insulin Mouse Serum assay kit offers superior specificity and sensitivity for a reliable quantification of insulin in serum and plasma samples. Built on a truly homogeneous protocol, the assay does not require any washing or complex extraction steps, leading to significant time savings over enzyme-linked immunosorbent assay (ELISA) methods.



Benefits

Specificity: only detects insulin and not insulin-related peptides

Sensitivity: a 70 pg/mL limit of detection allows for plasma or serum measurement of even the smallest insulin quantities

Ease of use: truly homogeneous technology with complete absence of washing steps

Sample economy: only requires 2 µL to 5 µL volume to achieve results

Validation: each assay is thoroughly validated on the industry's most common models and benchmarked against comparable technology prior to market release, ensuring that our kits will consistently deliver reliable and trustworthy data

TABLE OF CONTENTS

INSULIN MOUSE SERUM ASSAY KIT

Spotlight on Validation

Validation on normal mouse serum samples

Serum was collected from C57BL/6J mice that were either fasted overnight or fed normally. The insulin concentration was determined in each sample using the HTRF Insulin Mouse Serum kit reagents.

Validation on diabetic mouse plasma samples

Plasma was prepared with K3 EDTA after collection through cardiac puncture from male mice, either db/db diabetic or db/+ control, fasted overnight. The insulin concentration of each sample was determined using the HTRF Insulin Mouse Serum kit reagents.

Correlation with leading ELISA

HTRF Insulin Mouse Serum assay showed an excellent correlation (R^2=0.979 and R^2=0.913) to a leading absorbance and chemiluminescent ELISA both on sera with low titers and high titers of insulin.

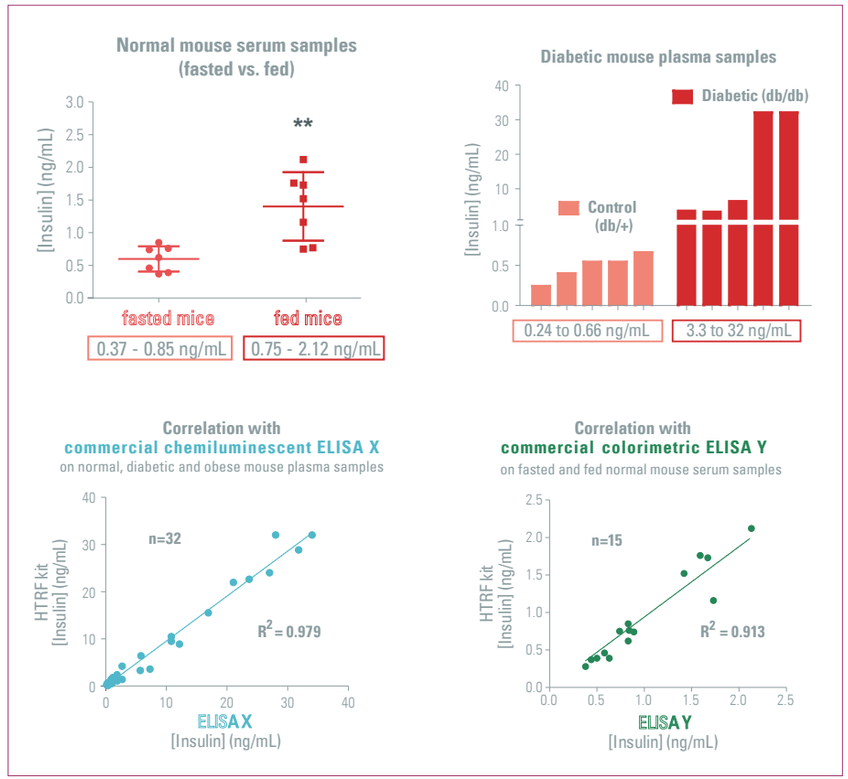


TABLE OF CONTENTS

Insulin High Range Assay Kit

High dynamic range for fewer sample dilutions

Highly concentrated samples often need to be diluted prior to insulin quantification in the assay, a cumbersome step that can lead to inaccuracies and lengthen the time to results. With no washing steps prior to insulin quantification, and a 4-log dynamic range, the HTRF Insulin High Range assay kit delivers the benefits of fewer sample dilutions and increased accuracy for straightforward and reliable quantification of insulin in highly concentrated samples.

Benefits

Extended dynamic range: 0.15 ng/mL to 150 ng/mL

Ease of use: truly homogeneous technology with complete absence of washing steps

Validation: each assay is thoroughly validated on the industry's most common models and benchmarked against comparable technology prior to market release, ensuring that our kits will consistently deliver reliable and trustworthy data

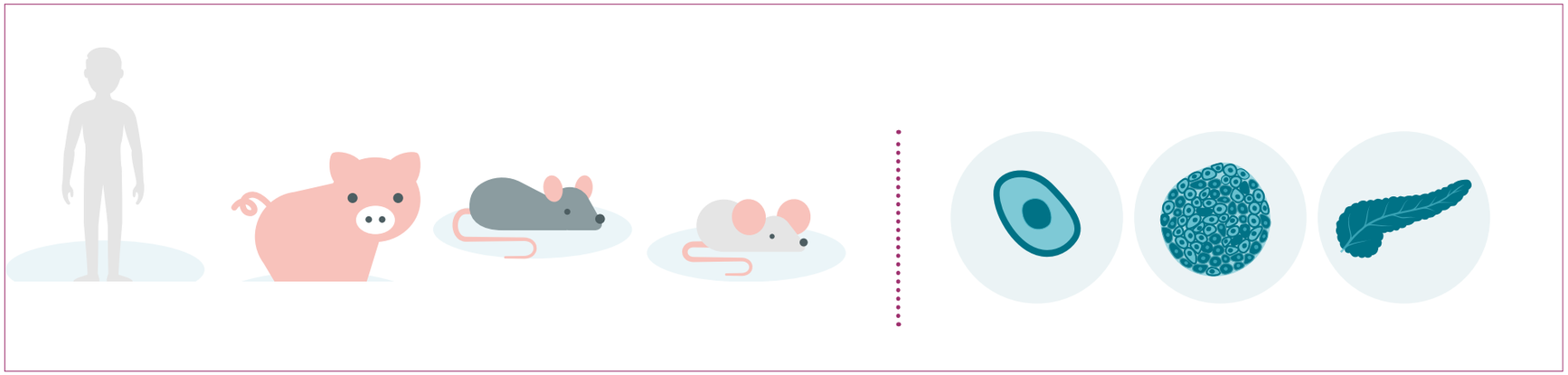


TABLE OF CONTENTS

INSULIN HIGH RANGE ASSAY KIT

Spotlight on Validation

Validation on rat and mouse β-cells:

MIN6 mouse β-cells and INS-1E rat β-cells were seeded in 24-well plates (400,000 cells/well in 500 µL medium) and cultured for 6 days in complete culture medium at 37°C - 5% CO_2. Before stimulation, cells were washed with Krebs-Ringer Bicarbonate buffer and a cellular quiescence step was performed in KRB buffer (without glucose) for 2 hours at 37°C. Cells were then stimulated with 250 µL of KRB buffer solutions containing increasing concentrations of glucose. After 10 and 30 minutes secretion for MIN6 cells or 2 hours secretion for INS-1E cells, cell supernatants* were collected and insulin concentration was measured using HTRF detection reagents. Each condition of treatment was measured in triplicate (error bars represent SEM).

*Samples were kindly provided by S. Dalle and M. Ravier's research team "Physiopathology of pancreatic beta-cells", IGF, Montpellier, France

Validation on Human pancreatic islets:

Pancreatic islets were isolated from three human pancreases (A, B, and C), placed in 24-well plates (50 islets/well, 3 wells/condition) and pre-incubated for 1h at 37°C in Krebs-Ringer Bicarbonate HEPES buffer containing 2.8 mM glucose. The islets were then incubated in the same solution for 1h at 37°C and the supernatant was collected to measure basal insulin secretion. The same islets were stimulated for 1h at 37°C with 16.7 mM glucose before collecting the supernatant to measure glucose-stimulated insulin secretion. At the end of the secretion test, the islets were lysed with a solution of acid-ethanol for 24h and the lysate was collected and centrifuged. The insulin concentration of supernatants* and lysates* was determined using the Insulin High Range kit reagents.

*Samples were generated at the "Laboratory of Cell Therapy for Diabetes", Institute for Regenerative Medicine and Biotherapy (IRMB), Montpellier University Hospital, France

Correlation with leading RIA

HTRF Insulin High Range assay showed an excellent correlation (R^2=0.911) to a leading RIA when tested on human pancreatic islet supernatants.

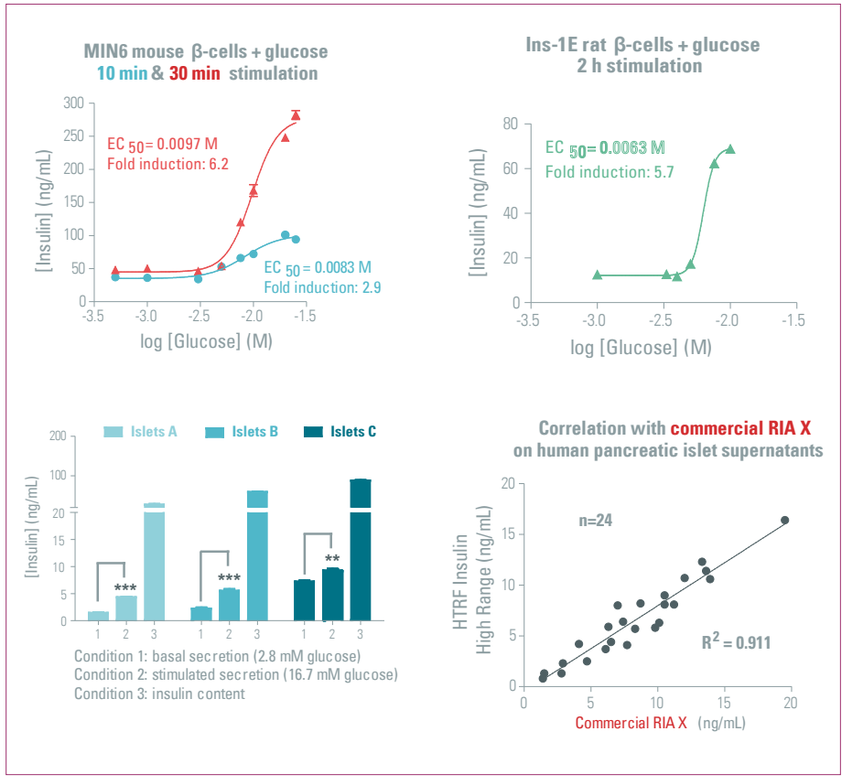


TABLE OF CONTENTS

Insulin Ultra Sensitive Assay Kit

Fast, accurate quantification of very low concentrations of insulin

One hurdle faced by researchers looking to quantify insulin is the high number of islets often required per assay. High assay sensitivity can significantly reduce the number of islets needed per well. With a sensitivity of around 4 pg/mL the HTRF Insulin Ultra Sensitive assay is designed with precious sample economy in mind, and allows for a significant decrease in the number of islets needed per tests.

Enhanced sensitivity also allows for the quantification of basal insulin levels from cells and insulin released from perfused mouse pancreas, models that typically generate low insulin levels.

Benefits

Enhanced sensitivity: 4 pg/mL

Ease of use: truly homogeneous technology with complete absence of washing steps

Validation: each assay is thoroughly validated on the industry's most common models and benchmarked against comparable technology prior to market release, ensuring that our kits will consistently deliver reliable and trustworthy data

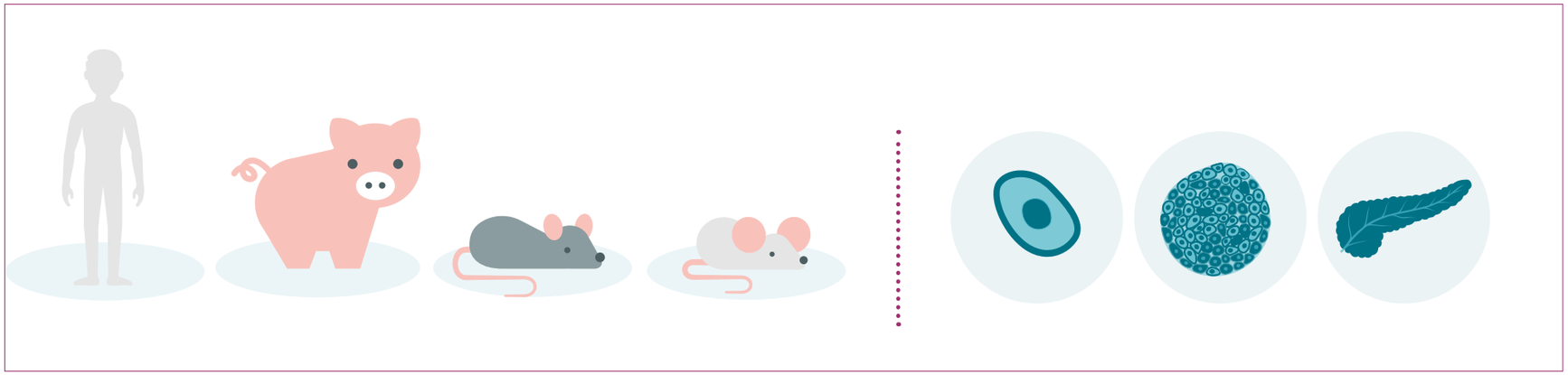


TABLE OF CONTENTS

INSULIN ULTRA SENSITIVE ASSAY KIT

Spotlight on Validation

Validation on mouse pancreatic islets:

Pancreatic islets were isolated from C57BL/6N male mice. The islets were dispensed in tubes with KRB buffer containing 2.8 mM or 16.7 mM glucose (5 islets/tube, 6 tubes/condition). After 10 minutes (left)or 30 minutes (right) incubation, supernatants* were collected and the insulin concentration was measured using the HTRF Insulin Ultra Sensitive assay kit reagents.

*Samples were kindly provided by S. Dalle and M. Ravier's research team "Physiopathology of pancreatic beta-cells", Institute for Functional Genomics (IGF), Montpellier, France

Correlation with leading RIA

HTRF Insulin Ultra Sensitive assay showed an excellent correlation (R^2=0.946) to a leading RIA on human pancreatic islet supernatants.

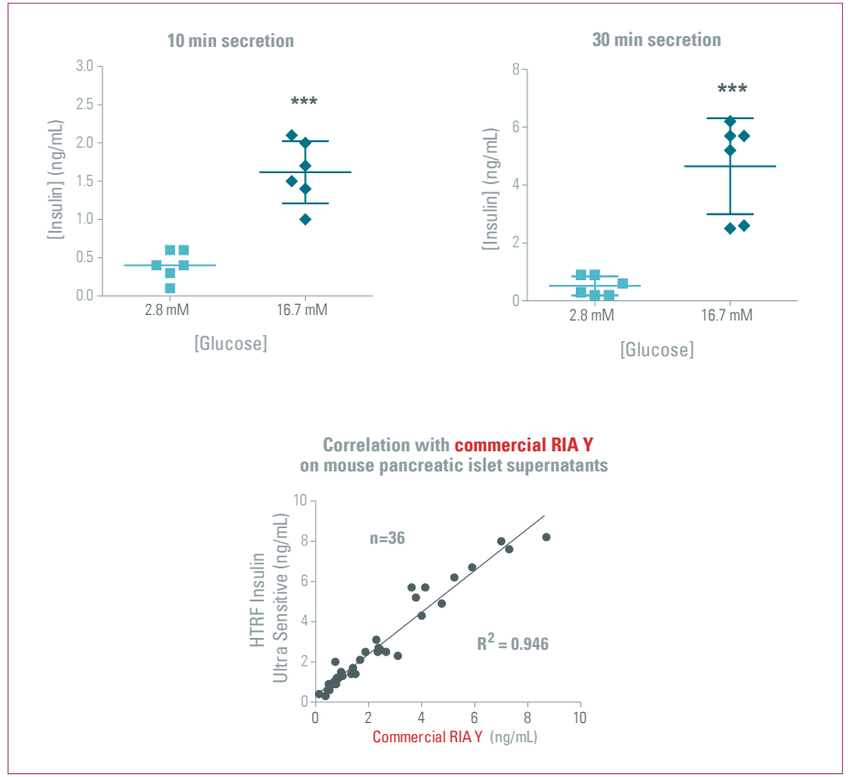


TABLE OF CONTENTS

Conclusion

The measurement of insulin using the HTRF platform offers tremendous advantages over the traditional ELISA technology. HTRF offers the specificity, sensitivity and accuracy of an ELISA, at a fraction of the time and sample volume it takes to get your results.

Designed for low sample consumption, the **Insulin Mouse Serum kit** is ideal for insulin quantification in precious serum samples. Designed for extended dynamic range, the **Insulin High Range kit** circumvents the need for dilution in highly concentrated samples. Finally, the **Insulin Ultra Sensitive kit** detects even the lowest concentration of insulin in hard-to-measure samples.

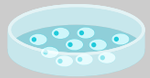
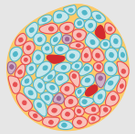
	IN VITRO	EX VIVO			IN VIVO
	β-cell line 	Isolated pancreatic islets 		Isolated perfused pancreas 	Plasma/Serum 
		Static experiment	Perfusion experiments		
Mouse	Insulin High Range e.g. Min6	Insulin Ultra Sensitive	Insulin Ultra Sensitive	Insulin Ultra Sensitive	Insulin Mouse Serum
Rat	Insulin High Range e.g. Ins-1	Insulin High Range	Insulin Ultra Sensitive	Insulin High Range	-
Human	Insulin Ultra Sensitive e.g. EndoC-βH1	Insulin High Range	Insulin Ultra Sensitive	Insulin High Range	-
Pig	Insulin High Range / Insulin Ultra Sensitive				-

TABLE OF CONTENTS

INSULIN QUANTIFICATION ASSAYS

List of Avalaible Insulin Assays and Related Assays

Product	Size	Part#
Insulin Mouse Serum kit	200 tests 5 x 200 tests	62IN3PEF 62IN3PEB
Insulin High Range kit	500 tests 10,000 tests	62IN1PEG 62IN1PEH
Insulin Ultra Sensitive kit	500 tests 10,000 tests	62IN2PEG 62IN2PEH
Glucagon kit	500 tests 10,000 tests	62CGLPEG 62CGLPEH
Glucagon Serum kit	200 tests 5 x 200 tests	62SGLPEF 62SGLPEB
Active GLP-1 kit	500 tests 10,000 tests	62GLPPEG 62GLPPEH

TABLE OF CONTENTS

DETECTION

Choosing the Right Plate Reader

Choosing the suitable microplate reader ensures you'll get an optimal readout. A multimodal reader gives your research the ideal flexibility and expands the field of possibilities by measuring a wide range of technologies.

Revvity offers multimode plate readers that are certified for use with HTRF technology. Certification involves rigorous testing of plate reader with HTRF technology to ensure optimal readout. HTRF assays are also compatible with multimode readers from other providers as well.

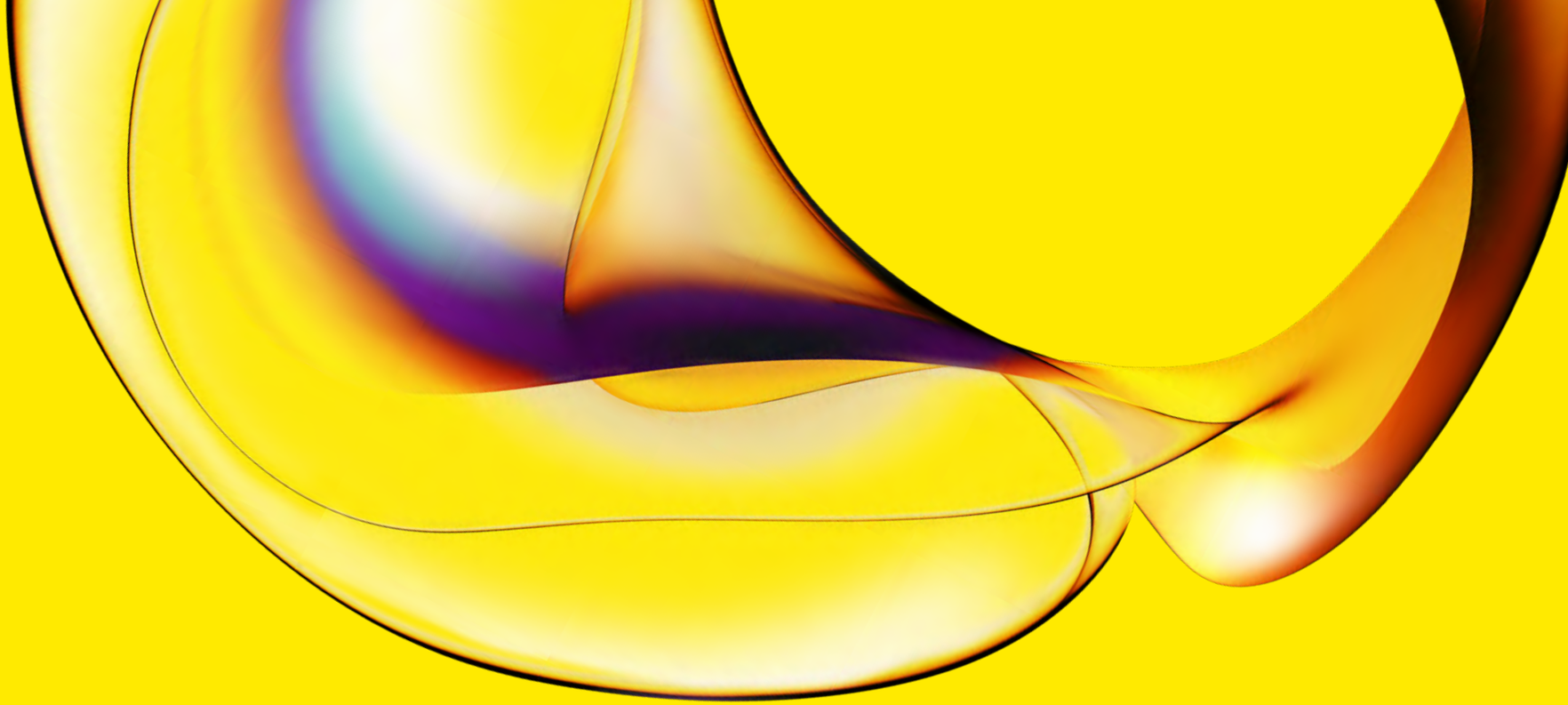
EnVision® Nexus™

Provides lightning speed and superior sensitivity across all established detection technologies with advanced options for ultimate performance. It is the next generation of high-throughput screening, ideal for your most demanding assays.

VICTOR® Nivo™

Packs all the latest major detection technologies into the industry's smallest benchtop footprint. The perfect microplate reader for everyday biochemical and cell-based assays.

TABLE OF CONTENTS



www.revvity.com

Revvity, Inc.
940 Winter Street, Waltham, MA 02451 USA
(800) 762-4000 | www.revvity.com

For a complete listing of our global offices, visit www.revvity.com

1185601