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Development of a Rapid HTRF Insulin Assay

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- **Direct measurement of insulin is necessary to further our understanding of insulin release**
- **The homogeneous HTRF Insulin assay proves to be suitable for HTS**

Introduction

When insulin secretion is not appropriately regulated it can lead to extensive metabolic problems which can result in diabetes mellitus[1]. Therefore, direct measurement of insulin is essential for the studies that will elucidate the mechanism of glucose stimulated release and the effects of dopaminergic modulators of insulin release[2].

Here we show the development of an insulin detection assay based on the homogeneous time resolved fluorescence (HTRF) detection method (Figure 1). Detection of this HTRF assay was assisted by the technology incorporated in to the PHERAstar *FS*, which is designed to optimize HTRF detection. This HTRF insulin assay provides quick and cost-effective results. The assay is further able to detect insulin from a variety of species. Performance was confirmed using insulin secreting cell lines and tissues, and tests indicate that this system will be suitable for detecting the effect of modulators on insulin secretion.

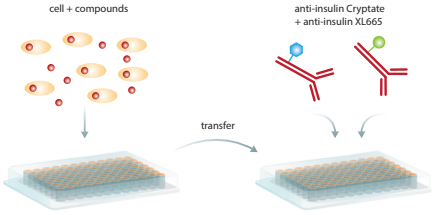

Fig. 1: Homogeneous HTRF Assay procedure. Secreted insulin in cells or supernatant is transferred to a new microplate, antibodies are added, incubated and read by the PHERAstar *FS* microplate reader.

Assay Principle

This HTRF insulin assay relies on two insulin binding antibodies (Figure 2). One antibody is labelled with a Europium cryptate donor and the second antibody is labelled with a near-infrared acceptor. When both antibodies are bound to insulin they are in close enough proximity for FRET (fluorescence resonance energy transfer) to occur. Because of the nature of the donor, a time delay before measurement start can be set that reduces transient interference from other assay components.

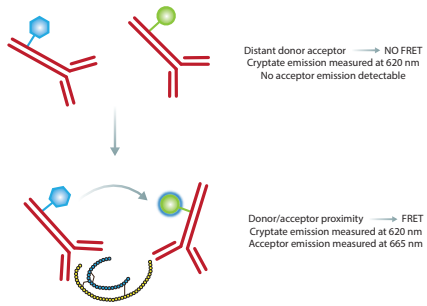

Fig. 2: Principle of the HTRF insulin assay. In the absence of insulin no FRET is occuring and only the donor will give a signal. The binding of both antibodies to insulin results in physical proximity, FRET occurs and acceptor emission can be detected. Ratiometric measurements reduce well to well variation.

Materials & Methods

- 96-well (half area) or 384-well, white plates (Greiner)
- Anti-insulin: Eu and anti-insulin: XL665 (Cisbio)
- Rodent insulin (ALPCO)
- Bromocriptine mesylate (Tocris)
- PHERAstar *FS* microplate reader

All other compounds were obtained from SigmaAldrich. For details on mouse pancreatic islet isolation, cell culture and the treatment of tissues and cells with insulin secretion modifying compounds please refer to Farino and Morgenstern *et al.*[2]

The HTRF insulin detection assay was performed by adding both anti-insulin antibodies to samples in a 1:1 ratio (total antibody volume: sample volume). After a 2 hour incubation at 25° C in pH 7 buffer the plate was read on a PHERAstar *FS*.

PHERAstar FS Instrument Settings:

Measurement type:	Time Resolved Fluorescence
Measurement mode:	Endpoint
Optic module:	HTRF (337/665/620)
Focal height:	adjust prior to test run
Integration start:	40 µs
Integration time:	100 µs
Excitation source:	flash lamp
Flashes/well:	100

Results & Discussion

The HTRF insulin assay was tested for the ability to detect insulin from a variety of species (Figure 3). The results indicate that the assay will be suitable for use with human cells and various animal models.

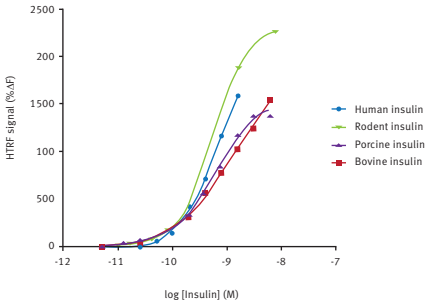

Fig. 3: Species cross-reactivity of antibodies. Data taken from Farino *et al.*[2]

No significant difference in HTRF signal were observed when insulin from humans, rodents, pigs or cows was tested across a range of concentrations from 0.01 – 10 nM (n = 3).

The suitability of this assay for high throughput screening was further assessed by the determination of the Z' – factor. Figure 4 shows the well-to-well variation of standards with different insulin concentrations.

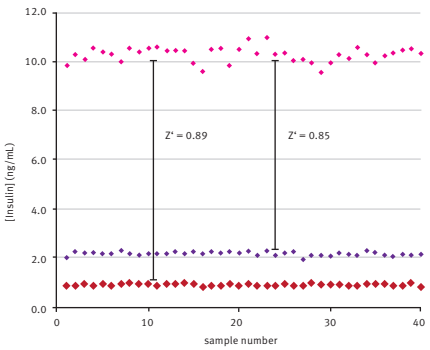

Fig. 4: Well-to-well variation of back calculated insulin concentrations. Data was first published in reference 3.

Results are based on 40 replicates and indicate that this assay is indeed suitable for HTS applications (Z'= 0.85, calculated between 2.5 ng/ml insulin and 10 ng/ml insulin samples).

To further illuminate the potential of this assay we tested the hypothesis that bromocriptine, a known dopamine D2/D3 receptor agonist might act directly on pancreatic islets. The results shown in Figure 5 indicate that bromocriptine can inhibit glucose stimulated insulin secretion in mouse pancreatic islets.

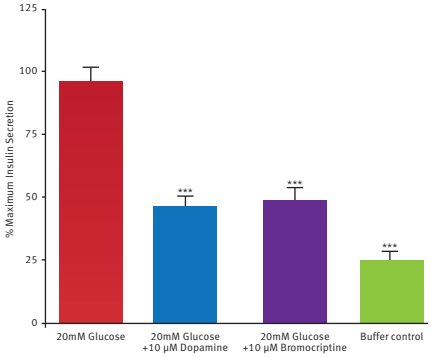

Fig. 5: Measurement of bromocriptine effect on insulin production in islets. Data are presented as % maximal insulin secretion in response to glucose. Bromocriptine inhibited insulin secretion by 67.4% ± 8.1% (n = 6). From Farino *et al.*[2]

Conclusion

The HTRF insulin assay is a homogenous assay that is straightforward and fast to perform. The results shown here indicate the suitability of this assay for medium/high throughput. Furthermore it can be performed at very low cost ($ 0.02 / sample)[2].

References

1. Kahn SE *et al.* (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes *Nature* **444**:840-846.
2. Farino ZJ and Morgenstern TJ *et al.* (2016) Development of a Rapid Insulin Assay by Homogenous Time-Resolved Fluorescence *PLoS ONE* **11**:e0148684.
3. Claret EJ *et al.* (2004) A high-throughput HTRF assay for human/rat insulin. *Cisbio International*. SBS Poster.

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