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 mellitus1. 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 release2.

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.

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

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.

Fig. 2: Principle of the HTRF insulin assay. In the absence of insulin no FRET is occurring 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.

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.
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.

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.

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