
INTRODUCTION
Insulin secretion triggered by glucose is potentiated by the activation of receptors coupled to G proteins (RCPGs), such as the GLP-1 (RGLP-1) or GPR-119 receptors. The agonists of these receptors have been widely studied for treating type 2 diabetes. In order to better characterize and compare the insulinosecretor and/or anti-apoptotic abilities of these receptor agonists, we have developed a new multi-parametric approach based on HTRF technology and which enables key parameters to be followed simultaneously: insulin secretion, the production of second messengers (cAMP), and the activation of survival transcription factors, such as CREB (cAMP-responsive element binding protein), by phosphorylation.

HTRF MULTI-PARAMETRIC APPROACH: MECHANISM OF ACTION OF MOLECULES WITH POTENTIAL THERAPEUTIC ACTIONS IN T2D

Principles & advantages of the HTRF® technology
Developed by Cisbio Bioassays, HTRF associates FRET (Fluorescence Resonance Energy Transfer) and time-resolved measurement (the delay between excitation and fluorescence signal measurement).
- FRET is based on energy transfer between 2 compatible fluorophores (the donor and acceptor) when they are located close to each other.
- Using resolved time means short-lived parasite fluorescence is eliminated: cell, compound, culture medium and plate self-fluorescence.
The HTRF technology requires no washing steps (homogeneous), and offers a sensitivity comparable to or better than standard methods (ELISA, Western Blot, Alpha-Lisa), as well as high assay reproducibility. Its simple implementation and fast acquisition of results are among its key advantages. As detection takes place in a microplate, only small sample volumes are necessary (10 µL).

Insulin secretion is potentiated more efficiently by activating the GPR119 receptor, compared to GLP1R receptor

The GPR119 receptor stimulates cAMP production more efficiently and for a longer time than the GLP1R receptor

The GPR119 and GLP1R receptors trigger CREB phosphorylation similarly

CONCLUSION
The HTRF technology, a simple and fast multi-parametric approach, opens up new avenues for the studying and characterizing compounds of therapeutic interest.
Clearly, dose-effect & kinetic effect are key parameters in diabetes physiopathology. Thus being able to integrate these different parameters in a multi-factorial analysis (different compound concentrations, different treatment times, several parameters analyzed) means that a precise, comprehensive study is possible from the same type of sample.

REAGENT REFERENCES
Blocking reagent (Cisbio 64KB1AAC)
CREB p-S133 kit (Cisbio ref.64CREPEG)
cAMP dynamic 2 kit (Cisbio ref.62AM4PEB)
Insulin kit (Cisbio ref.62INSEPBC)
Lysis Buffer (Cisbio 64KL1FDF)
Exendine-4 (H87-30, Bachem)
AS1269574 (4177, Tocris)