

Cisbio's HTRF[®] cortisol assay performed on the PHERAstar

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Introduction

Cortisol is a corticosteroid hormone present in many metabolic processes, inducing key enzymes of carbohydrate, fat and protein metabolism. Cortisol also acts as an anti-inflammatory and immunosuppressor. One way to create this important hormone is by the reduction of cortisone by NADPH dependent 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta-HSD1).¹ This enzyme can be found in several tissues but it is mostly present in liver and fat cells.¹

Recently Cisbio developed an assay to determine the activity of 11 beta-HSD1 using HTRF[®] technology. HTRF[®] (Homogeneous Time-Resolved Fluorescence) is based on TR-FRET (time-resolved fluorescence resonance energy transfer) between a Eu³⁺ cryptate (donor) and a second fluorescent label (acceptor) d2.²

BMG LABTECH's PHERAstar (Figure 1) is a multifunctional plate reader that combines rapid plate reading necessary for high throughput screening (HTS) with the enhanced performance and sensitivity needed to read small fluid volumes. The PHERAstar has been designed to read all HTS detection modes (fluorescence intensity, time-resolved fluorescence, fluorescence polarization, luminescence, AlphaScreen[™] and absorption) in all plate formats up to 1536 wells. To meet the HTRF[®] requirements the PHERAstar uses a unique HTRF[®]-specific optical module that can measure two emission signals simultaneously.

Materials & Methods

Instruments

- BMG LABTECH's PHERAstar, Offenburg, Germany
- HTRF[®] optical module (excitation: 337 nm, emission A: 665 nm and emission B: 620 nm), Offenburg, Germany
- Cisbio's HTRF[®] Cortisol assay (Cat. no. 62CO2PEB) Bagnols, France



Fig. 1: BMG LABTECH's high-end microplate reader PHERAstar.

Cortisol standard curve

10 µL of cortisol standards (serial dilution) and 5 µL of each HTRF[®] conjugate (anti-cortisol cryptate and cortisol-d2) were dispensed into the wells of a black 384 small volume microplate from Greiner. The plates were measured using the HTRF[®] module after both two hours and 16 hours of incubation.

Biochemical assay

2 µL of 11beta-HSD1 microsomal preparation (0.1 mg/mL) in Tris 20 mM EDTA 5 mM buffer (pH = 6), 6 µL of Tris 20 mM, EDTA 5 mM buffer (pH = 6) containing cortisone 266 nM and NADPH 333 µM and 2 µL of inhibitor (carbenoxolone and glycyrrhetic acid) at different concentrations in Tris 20 mM EDTA 5 mM buffer were dispensed into the wells. After 2 hours of incubation at 37°C, 5 µL of each HTRF[®] conjugate (anti-cortisol cryptate and cortisol-d2) were added. The plates were incubated for another 2 hours at room temperature before reading on the PHERAstar.

The signal is expressed in DeltaF in % (DF%)

$$DF\% = \frac{\text{Ratio}_{\text{pos. control}} - \text{Ratio}_{\text{neg. control}}}{\text{Ratio}_{\text{neg. control}}} \times 100$$

where Ratio = (Signal at 665nm / Signal at 620nm) x 10⁴ and pos.control = positive control and neg. control = negative control.

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Results and Discussion

Figure 3 shows cortisol titration curves at different incubation times.

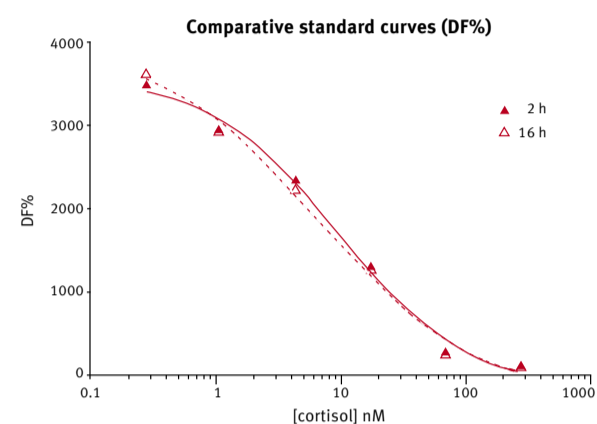


Fig. 3: Cortisol titration curves recorded on the PHERAstar after 2 hours and 16 hours incubation

This data demonstrates that with increasing cortisol concentration the anti-cortisol cryptate is displaced proportionally resulting in a decreasing signal curve.

Table 1: EC₅₀ values of cortisol standard curve after different incubation times

Incubation	EC ₅₀ µM
2 h	5.38
16 h	6.16

Furthermore, it is shown that an incubation time of 2 h is sufficient. Longer incubation times will not lead to different results regarding DF% and EC₅₀ values (Figure 3 and Table 1).

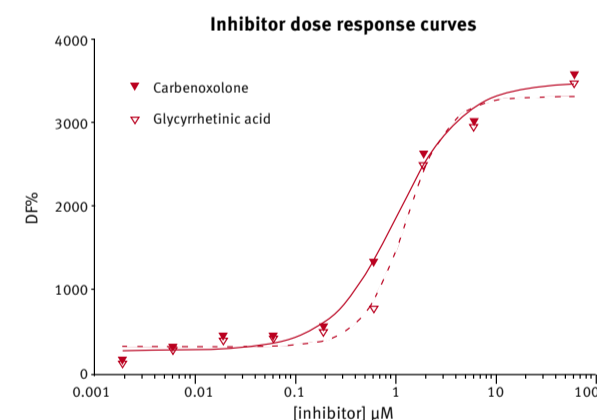


Fig. 4: Inhibitor dose response curve.

Inhibitor response curves with the known inhibitors carbenoxolone and glycyrrhetic acid are graphed in Figure 4. The inhibitors show similar behaviour, which is also expressed in similar IC₅₀ values (Table 2). The IC₅₀ values of these known inhibitors are comparable with literature results indicating that the assay and instrument setup work properly.

Table 2: IC₅₀ values of 11 beta-HSD1 inhibitors calculated from dose response curves

Inhibitor	IC ₅₀ µM
Carbenoxolone	1.03
Glycyrrhetic acid	1.17

Assay Principle

The cortisol assay is a monoclonal antibody based competitive assay (Figure 2). It is run in two steps. After the dehydrogenase reaction is finished (stimulation step), anti-cortisol cryptate (donor) and d2 labeled cortisol (acceptor) are added to the reaction mix. The anti-cortisol cryptate and the d2 labeled cortisol will bind to each other leading to a high HTRF[®] signal. Cortisol built during the enzymatic reaction will compete with d2-labeled cortisol for the binding to the cryptate conjugate, resulting in a loss in HTRF[®] signal (detection step).

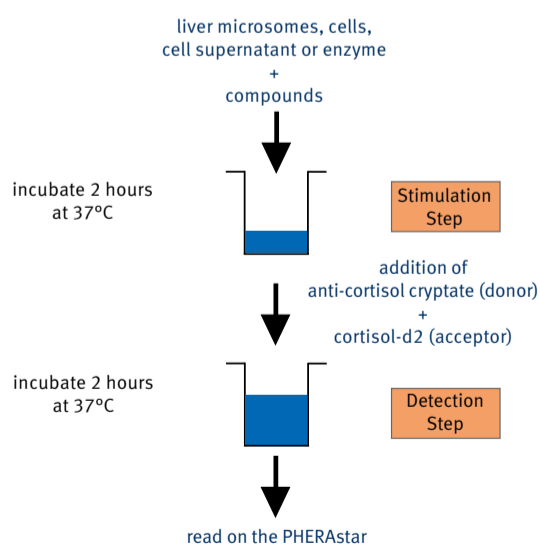


Fig. 2: Principle of Cisbio's cortisol assay.³

Conclusion

The cortisol assay allows fast and efficient determination of cortisol in complex samples such as serum and whole cells. Screening for both active 11 beta-hydroxysteroid dehydrogenase type 1 and its inhibitors is also simple and effective using this homogeneous assay. The PHERAstar in combination with the optimized HTRF[®] optical module is the ideal tool to run HTRF[®] assays. The PHERAstar's optical design provides for outstanding sensitivity and accuracy in fluorescence and luminescence assays; moreover, the simultaneous dual measurement minimizes the read time for assays.

References

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