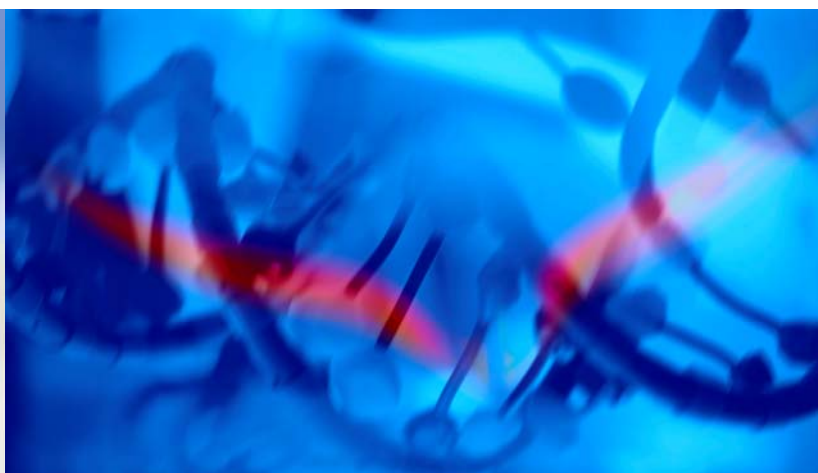
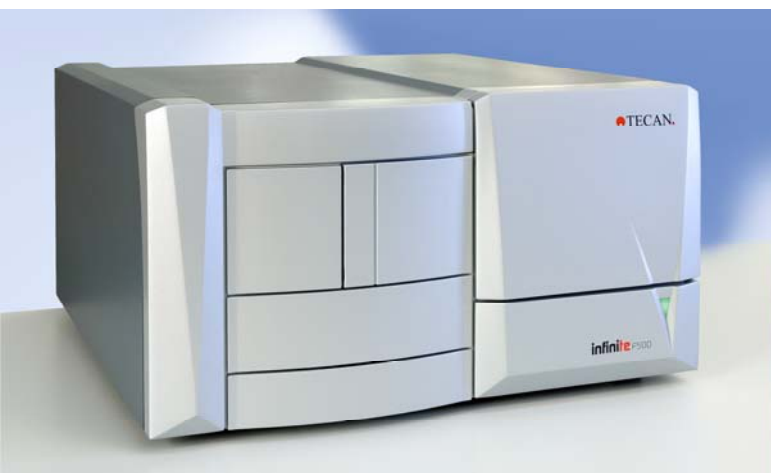


PolarScreen Red™ (Invitrogen) Glucocorticoid Receptor Assay

Tecan Infinite™ F500, Fluorescence Polarization



Introduction

The Glucocorticoid Receptor

The Glucocorticoid Receptor (GR) belongs to the important superfamily of ligand-activated, intracytoplasmatic transcription factors, the so called Nuclear Receptors (NR). NRs mediate cellular responses to a broad range of small molecular weight, non-peptide signals, including endogenous hormones and metabolites as well as xenobiotic compounds (1-3).

How does the Glucocorticoid receptor work?

An adequate ligand, for example cortisol, passes the cellular membrane and binds to the cytoplasmatic GR. Due to this binding the GR releases some heat shock proteins, followed by the release of heat shock chaperones and this yields the free cortisol-receptor subunits. Anyway, these subunits are translocated into the nucleus and work as transcription factors (zinc finger system). The following biological response is cell line specific (1,2). The study of NRs and their ligands is an important field in the development of novel therapeutics to fight especially hormone linked diseases.

Assay description

Invitrogen has developed a variety of so called *PolarScreen™ Nuclear Receptor Assays*, for example the Glucocorticoid Receptor Competitor Assay, in order to offer a simple and reliable method in the research of NRs (3).

All these assays include a specific nuclear receptor, a fluorescent Fluoromone ligand and an optimized buffer system; they all work as a competitive system. In the case of NR-Fluoromone ligand binding a high polarization is the consequence. If the respective compound of interest (any possible ligand) displaces the Fluoromone ligand from the complex, the polarization value is lowered to a certain degree. This shift in polarization let one draw conclusions concerning the relative affinity of the respective test compound for the NR.

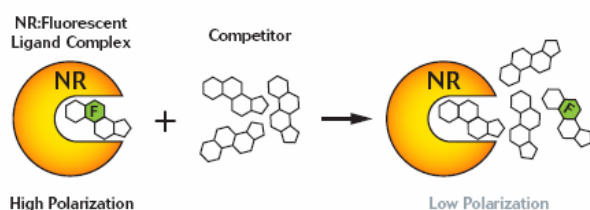


Figure 1: Scheme of PolarScreen™ NR Assays

As mentioned, the Glucocorticoid Receptor Competitor Assay is an assay to estimate the affinity of some test compounds for the human Glucocorticoid Receptor. This system works with a fluorescent glucocorticoid as ligand (Fluoromone™ GS Red). In the presence of an effective test compound GS Red is displaced resulting in decreasing polarization. The system works with red fluorescence to minimize background interferences (3).

Material and methods

Instrument

Tecan Infinite™ F500 filter-based microplate detection system

Microplates

384 Flat bottom Black Polystyrol small volume micro plates (Greiner Bio-One)

Reagents and Assay Performance

Reagents

PolarScreen™ Glucocorticoid Receptor Competitor Assay, Red (Invitrogen P2893). DMSO, dexamethasone diluting buffer (2.5% DMSO in water), Dexamethasone (Sigma, D4902)

Reagent preparation

The assay buffer was prepared according to the manufacturers assay protocol and was used for dilution of Fluoromone™ GS Red and Glucocorticoid Receptor stock solutions. Preparation of dexamethasone ready-to-use solutions: 1 mM dexamethasone DMSO stock solution was initially diluted in DMSO to a range of different concentrated dexamethasone solutions (150 – 0.0015 μ M). These solutions were, further on, diluted in H₂O (1/10) and finally, diluted 1/5 with dexamethasone diluting buffer prior to use.

Assay protocol

5 μ l 3 nM Fluoromone™ GS Red were mixed with 5 μ l dexamethasone ready-to-use solution. The reaction was initiated by adding 5 μ l 12 nM Glucocorticoid Receptor Working Solution (GR WS). After incubation for 4 h at RT, the fluorescence polarization was measured. For reaction blank, a mix of 5 μ l 12 nM GR WS, 5 μ l assay buffer and 5 μ l dexamethasone dilution buffer was used. The measurements were carried out in five replicates.

Measurements

G factor calculation

G factor calculation was performed using a well containing 5 μ l of 3 nM GS Red, 5 μ l assay buffer and 5 μ l dexamethasone dilution buffer. Reference polarization value: 100 mP, reference blank = same as reaction blank.

Measurement settings

Measurement 1	
Ex wavelength	530 nm
Ex bandwidth	20 nm
Em wavelength	590 nm
Em bandwidth	20 nm
Lag time	0 μ s
Integration time	40 μ s
Number of reads	10*
Optimal gain	94
Manual z-position**	calculated from well

Table 1: Fluorescence Polarization measurement parameters for Infinite™ F500

Results and Discussion

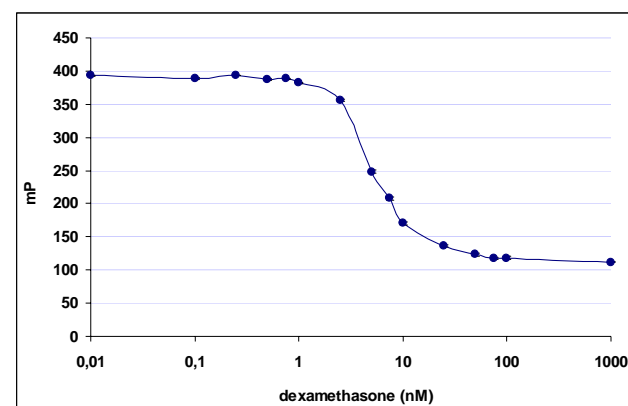


Figure 2: Glucocorticoid receptor assay - Titration with the competitor test compound dexamethasone

The Glucocorticoid Receptor Competitor Assay utilizes the concept of the fluorescence labeled ligand binding to a large receptor. Such receptor-ligand complexes always display high FP values unless the labeled ligand is displaced from the receptor by a compound, competing for the same receptor binding site. In that case, the polarization decreases and the shift in polarization value is used to determine the affinity of the screening compound for the particular receptor. The greater the affinity of the ligand of interest for the receptor, the lower the polarization value.

In the present measurements, the reaction was started by addition of GR to a fluorescent glucocorticoid ligand in the presence of the competitor test compound dexamethasone. As expected, increasing concentrations of dexamethasone could subsequently totally prevent the formation of the GS Red/GR complex indicated by the change in the FP signal from 390 to 100 mP (see Figure 1). The inhibition constant, IC₅₀, of ~ 5 nM could be determined and is in accordance with the specification criteria defined by Invitrogen. The IC₅₀ refers to the dexamethasone concentration which results in a half-maximum shift in polarization value.

Conclusion

The obtained measurement results clearly indicate that the Infinite™ F500 can easily be optimized to perform fluorescence polarization based receptor-ligand assays, such as described here with the Glucocorticoid Receptor Competitor Assay.

Literature

- [1] Bran M., Necela and John A. Cidlowski., A Single Amino Acid Change in the First Zinc Finger of DNA Binding Domain of the Glucocorticoid Receptor Regulates Differential Promoter Selectivity, *J.Biol.Chem.* Vol 279, 2004, Issue 38, p.39279-39288
- [2] Stevens A. et al., Dissociation of steroid receptor coactivator 1 and nuclear receptor corepressor recruitment to the human glucocorticoid receptor by modification of the ligand-receptor interface: the role of tyrosine 735, *Mol. Endocrinology*, 2003, 17(5):845-859
- [3] Invitrogen homepage: <http://www.invitrogen.com>

List of abbreviations

DMSO	dimethylsulfoxid
GR	glucocorticoid receptor
NR	nuclear receptor
FP	fluorescence polarisation

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