Technical Demo #3

Recombinant Human Glucocorticoid Receptor in a Fluorescence Polarization-Based Ligand Binding Assay

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Glucocorticoid receptor (GR), a member of the nuclear receptor superfamily, is an important drug target for a range of disorders including inflammation, autoimmune diseases and cancer. To address these, we have developed a robust HTS assay using a consistent source of the recombinant receptor. Human GR was overexpressed in insect cells and a partially purified fraction was obtained that exhibited high affinity binding to dexamethasone (K_d ~ 4.5 nM). A homogenous competitive ligand binding assay was developed using a fluorescent glucocorticoid (Fluormone™ GS1) that bound specifically to hGR with a subnanomolar K_d; an increase of greater than 200 millipolarization (mP) units was attained at saturating hGR. A series of GR ligands was screened for competition with Fluormone™ GS1 and the relative binding affinities (RBA) compared with RBAs from radioligand assays. The Z´-factor for the assay was determined to be >0.5 in 96-well plates, indicative of a high quality, quantitative HTS assay.