

Application Examples for the TR-FRET assay system

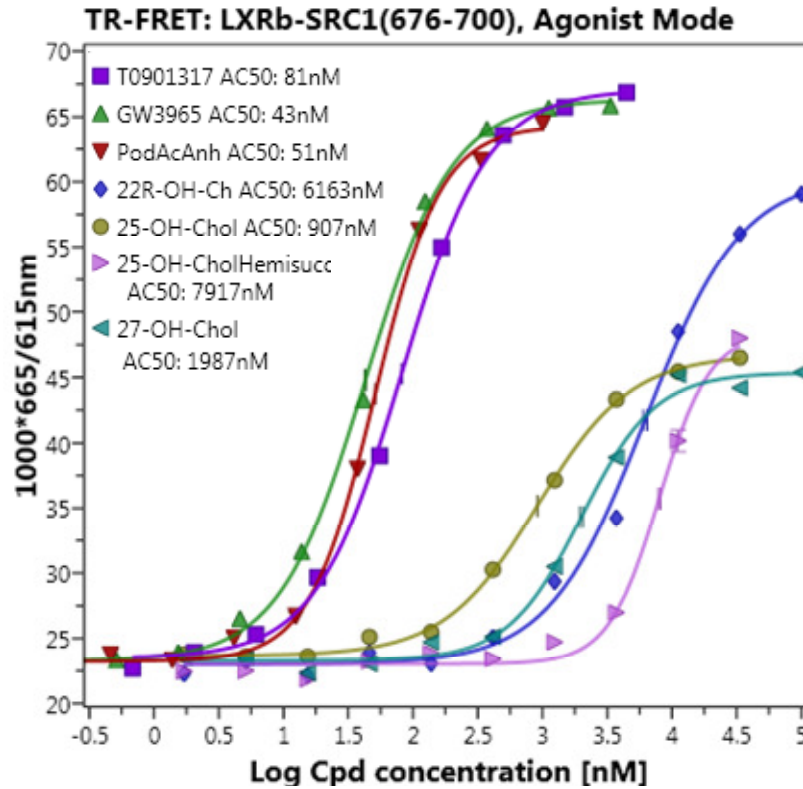


Figure 1

Here we show some synthetic agonists and some natural ligands in a TR-FRET assay with the ligand binding domain of the Liver X Receptor beta (NR1H2). T0901317, Podocarpic Acid Anhydrid (APD) and GW3965 are full LXRbeta agonists, whereas the tested hydroxycholesterols are partial modulators of LXRbeta in this biochemical assay.

The graph has been generated with an assay analysis software system developed by Hölle & Hüttner AG (<http://www.h-net.com/index.php/leistungen/software-projekte/195-phast>), defined by Phenex AG.

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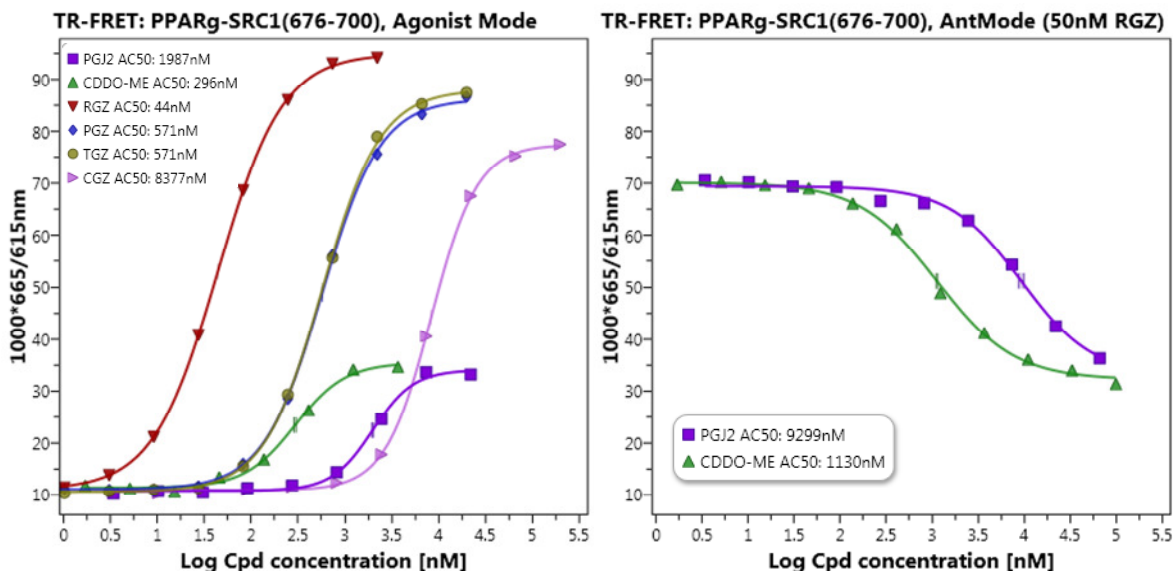


Figure 2

On the left graph we show a group of Thiazolidinediones (TZDs) and two other compounds in an Agonist Mode TR-FRET assay with the ligand binding domain of the Peroxisome Proliferator-Activated Receptor gamma (NR1C3). On the right graph we show 15-Deoxy-Delta-12,14-prostaglandin J2 (PGJ2) and the Triterpenoid CDDO-Me in an Antagonist Mode TR-FRET assay. In Antagonist Mode assays, the reference compound is added into the assay in an intermediate concentration and an antagonistic compound is expected to decrease the baseline signal by displacement of the reference compound – this can be observed for PGJ2 and CDDO-Me. The curves show that the compounds have different potencies and different efficacies. The partiality of PGJ2 and CDDO-Me is supported by the Antagonist Mode assay, where the compounds reduce the elevated baseline signal to the maximum signal the compounds induce in the Agonist Mode assay.

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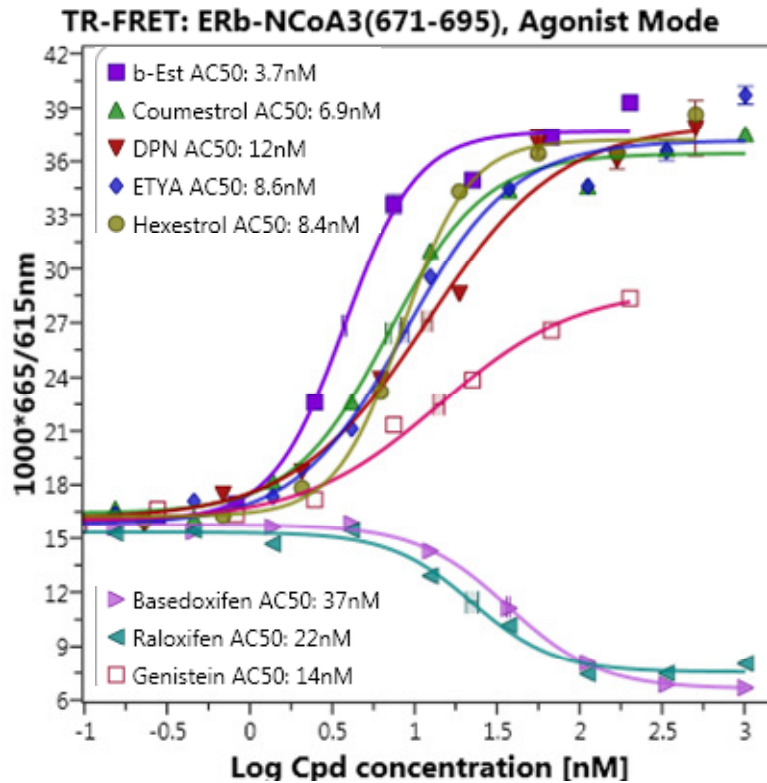


Figure 3

Here we show some more compounds in a TR-FRET assay with the ligand binding domain of the Estrogen Receptor beta (NR3A2).

ERbeta shows some constitutive activity in this TR-FRET assay with the NCoA3(671-695) peptide, therefore we can see already in the Agonist Mode assay, that Basedoxifen and Raloxifen are full ERbeta antagonists.

Genistein is the only partial ERbeta modulator used in this assay, b-Est, Coumestrol, DPN, ETYA and Hexestrol are full agonists at ERbeta in this biochemical assay.

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