

Studying the Affinity of Different Ligands to Estrogen Receptor- α with Thermo Scientific Appliskan[®]

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Abstract

This application note describes the performance of the Thermo Scientific Appliskan in the homogeneous Estrogen Receptor- α Competitor Assay based on fluorescence polarization. 17 β -estradiol and tamoxifen were used as test compounds to study their affinity to estrogen receptor- α .



Introduction

Fluorescence polarization (FP) is used to study molecular interactions. The molecular size affects the rotation speed of the fluorescent molecule. The larger the molecule is, the slower it rotates. And the slower the molecule rotates during its fluorescent lifetime, the more the emission light stays polarized. Therefore, the larger the molecular size of the fluorescent complex is, the higher the polarization value (mP) will be. The binding or dissociation reactions lead to changes in the sizes of the molecular complexes so that molecular interactions can be studied by detecting changes in the polarization value.

Estrogens are a group of steroid compounds functioning as the primary female sex hormone.

Estrogens act on target tissues by binding to estrogen receptors and that way activate or inactivate signaling pathways in cells. Cell proliferation is one of estrogens' natural roles, but estrogens can also stimulate the development of breast cancer by causing uncontrollable cell growth.

The estrogen receptor assay can be used to study the affinity of different ligands to estrogen receptors (ER). Also, the effect of any chemical on the binding of the ligand to ER can be studied. ER α is well-known as a drug target for the treatment of breast cancer. Molecules which block the action of estrogens by binding to the receptor or directly to estrogen can be used to prevent the negative effects of estrogen. In this assay 17 β -estradiol and tamoxifen were used as test compounds. 17 β -estradiol is the main endogenous human estrogen and tamoxifen is a drug molecule used in breast cancer treatment. The principle of the assay is presented in Picture 1.

Materials and Methods

The Estrogen Receptor- α Competitor Assay (Green) kit (# P2698, Invitrogen, USA) was used to study the affinity of the test compounds to ER α . The 10 mM stock solutions of 17 β -estradiol and tamoxifen (# E8875 and #T5648, Sigma, USA) were made in DMSO. The dilution series of the test compounds were made in the ES2 Screening Buffer of the kit. The concentration range of

the dilution series of 17 β -estradiol in reaction was from 2 to 125 nM and the range of tamoxifen in reaction was from 50 to 2500 nM. Black 384 square well microplates (# 8255, Thermo Fisher Scientific, Finland) were used in the assay.

50 μ l of the ER/Fluormone solution was added per well. The concentrations of ER and Fluormone in the solution were 30 nM and 2 nM, respectively. 50 μ l of the dilutions of the test compounds, 3 replicas of each, were mixed with the ER/Fluormone complex. Assay controls were done according to the kit instructions. The plate was incubated in dark at room temperature for 2 hours. Fluorescence polarization was measured with Thermo Scientific Appliskan using a 485 nm (HBW 10 nm) excitation filter and 535 nm (HBW 20 nm) emission filters, all with polarizers. The result calculations were performed with SkanIt Software 2.3 for Appliskan.

Results

All polarization values were corrected using the G-correction calculation of SkanIt Software. The G-value corrects the possible optical differences deriving from using two separate emission filters and separate polarizers. The calibration curves of 17 β -estradiol and tamoxifen are presented in Picture 2.

As the results show, 17 β -estradiol and tamoxifen compete with fluorescein labeled estrogen on a clearly different concentration range. A

higher concentration of tamoxifen than 17 β -estradiol is required to replace the labeled estrogen, as was expected, as the binding affinity of 17 β -estradiol is stronger. Both of the test compounds showed a concentration-dependent effect on replacing the labeled estrogen from the estrogen receptor. The ED50 values of the test compounds are presented in Table 1.

Table 1. The ED50 values of the test compounds

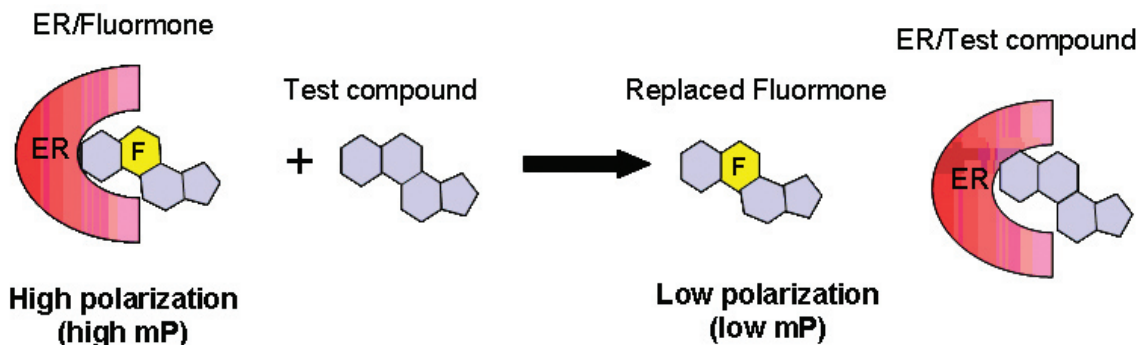
Test compound	ED50 (nM)
17 β -estradiol	13
Tamoxifen	520

Discussion

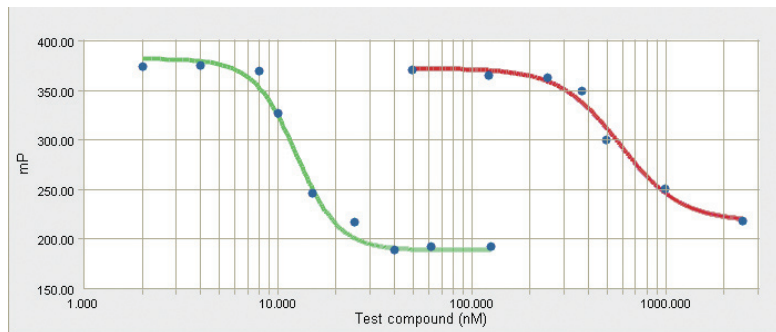
Fluorescence polarization assays are homogeneous and therefore relatively fast and easy to perform. Also, the FP assay development is quite easy as the natural reaction of the studied compounds is only slightly affected by the procedure. The Thermo Scientific Appliskan together with the Estrogen Receptor- α Competitor Assay kit can be used for studying the binding of any potential estrogen receptor ligands. The information about the affinities of different ligands can be used for drug discovery purposes.

References

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- Zou, A., Marschke, K.B., Arnold, K.E., Berger, E.M., Fitzgerald, P., Mais, D.E., Allegretto, E.A., 1999. *Estrogen Receptor β Activates the Human Retinoic Acid Receptor α -1 Promoter in Response to Tamoxifen and Other Estrogen Receptor Antagonists, but not in Response to Estrogen. Molecular Endocrinology* 13 (3), 418-430



Picture 1. The principle of the Estrogen Receptor- α Competitor Assay. ER α is first mixed with Fluormone (F), a fluorescein-labeled estrogen, to form the ER α /Fluormone complex. The complex is then mixed with the test compound on a microplate. The test compound competes with Fluormone for the binding to ER α . The more there is of the test compound, the more it replaces Fluormone in the ER α /Fluormone complex. The changes in the polarization value (mP) reflect the amount of test compound bound to ER α . As the concentration of the test compound increases, the polarization value decreases.



Picture 2. The calibration curves of the test compounds. The polarization values (mP) are plotted against the test compound concentrations (nM). The green curve represents 17 β -estradiol and the red curve tamoxifen.

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