Technical Demo #1

Development of Nonisotopic High-Throughput Screening Assay for Estrogen and Androgen Receptor Ligands

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Estrogen receptors and an androgen receptor are ligand-activated intracellular transcriptional regulators that are members of the steroid-thyroid hormone nuclear receptor superfamily.

In this study we have developed a nonisotopic high-throughput receptor-based assay to_ estimate the binding affinity (likely dissociation constants of receptor-chemical complexes) of chemical substances for receptors. The assay method is very simple (only dispense and incubate), and doesn't need a special equipment. The value of binding affinity between chemical substances and those receptors was able to be estimated easily by the calculation using absorption data, concentration of chemicals and that of receptors. The value of binding affinity is more an useful measure of inhibitory potency than the IC_{50} value, and it can be obtained by an only single measurement at a concentration of the chemical. The values of binding affinity between several chemicals and ER_were obtained in DES(2.3nM), genistein(61nM), zearalenone (142nM),and those for the androgen receptor were founded in mibolerone (3.5nM),_p,p'-DDD(5390nM), p,p'-DDE (3534nM), p,p'-DDT(3714nM).

The features of our method are the following

1. It can distinguish the denatured reaction of these receptors by chemicals from the binding reaction between the receptor and chemicals.
2. Using a binding affinity, it is possible to calculate the IC_{50} of chemicals for receptors, too.
3. It is able to measure many samples simultaneously, so it accomplish the high-throughput screening of chemicals.
4. It will be available to apply on the metabolic activation test using rat liver S9.

This assay method should serve as an useful tool of a prescreening test which is based on the in vitro-in vivo strategy to assess the effects of chemicals on the endocrine function