Development of Fluorescence Polarization Receptor Binding Assay for Rapid Screening of Endocrine Disrupters

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For the rapid screening of environmental endocrine disrupting chemicals (EDCs), a homogeneous fluorescence polarization receptor assay (FPRA) was developed using estrogen receptor and fluorescein-labeled estrogen tracer. Cytosolic estrogen receptor was prepared from rat uterus, and its quality was evaluated by radioreceptor binding assay using 3H-estradiol radiotracer. Fluorescein-labeled estrogen tracer was synthesized by labeling ethylenediamine fluoresceinthiocarbamyl (EDF) to 6-ketoestradiol-6-(o-carboxymethyl)oxime using the EDC coupling reaction and purified using the silica gel TLC plate. The resulting estrogen-6-EDF (E-6-F) tracer was evaluated by antibody binding activities.

The optimum condition of FPRA was investigated by studying physico-chemical factors influencing sensitivity of standard curve of estradiol. After characterization of receptor binding activities with estrogenic chemicals (diethylbesterol and tamoxifen) and androgenic chemicals (methyltestosterone and flutamide) using FPRA, relative binding activities of various EDCs was investigated.

This FPRA system, which needs no separation step between free tracer and receptor-bound tracer, takes 20 minutes for 10 samples using photo check mode of fluorescence polarization analyzer.