Assays Using Fluorescent Polarization

Reference	Bolger et al. (1998)	Hanioka et al. (1999)	Hashimoto et al. (2000)
Preparation of receptor			
Species and subtype of receptor	human ERα	human ERα	human ERα
Source of receptor	PanVera	n.p.	n.p.
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	recombinant
Buffer for assay of receptor	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
Protein concentration	n.p.	n.p.	n.p.
Competitive binding assay			
Ligand used	ES2	ES2	ES2
Concentration of estrogen	1 nM	1 nM	1 nM
Fluorescent ligand	FES1 ERα 13 nM, ERβ 10 nM	FES1 ERα 13 nM, ERβ 10 nM	FES1 ERα 13 nM, ERβ 10 nM
Concentration of fluorescent ligand	2 nM	2 nM	2 nM
Solvent used to dissolve competing ligand	10 mM ethanol	10 mM ethanol	10 mM ethanol
Concentration range of competing ligand	200 µM	200 µM	200 µM
Number of replicates	3	3	3
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	60 min	60 min	60 min
Temperature of incubation	room temp	room temp	room temp
Data calculations			
Fluorescence anisotropy	490 nm excitation; 530 nm emission filter	360 nm excitation; 535 nm emission filter	360 nm excitation; 530 nm emission filter
Program or method used for calculating data	Anisotropy converted to fraction bound	Nonlinear least squares regression	Anisotropy converted to percent inhibition
Data plotted as	Ligand bound=fraction bound x ligand conc.	Millipolarization vs. conc. of chemicals	Percent inhibition vs. competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	K_d	IC_{50}	Percent inhibition
Calculation of RBA	Nonlinear least squares regression	Nonlinear least squares regression	n.a.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Fluorescent Polarization

Reference	Nikov et al. (2000)	Nikov et al. (2001)	Parker et al. (2000)
Preparation of receptor			
Species and subtype of receptor	human ERα and ERβ	human ERα and ERβ	human ERα and ERβ
Source of receptor	PanVera	PanVera	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	n.p.
Buffer for assay of receptor	100 mM $KaPO_4$ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide	100 mM $KaPO_4$ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide	100 mM $KaPO_4$ pH 7.5; 100 µg/ml bovine gamma globulin, 0.02% sodium azide
Protein concentration	n.p.	n.p.	n.p.
Competitive binding assay			
Ligand used	ES2	ES2	ES2
Concentration of estrogen	1 nM	1 nM	1 nM
Fluorescent ligand	FES1	FES1	FES1 ERα 13 nM, ERβ 10 nM
Concentration of fluorescent ligand	n.p.	n.p.	n.p.
Solvent used to dissolve competing ligand	8 mM ethanol	8 mM ethanol	n.p.
Concentration range of competing ligand	n.p.	n.p.	n.p.
Number of replicates	n.p.	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	60 min	60 min	2 hours
Temperature of incubation	room temp	room temp	room temp
Data calculations			
Fluorescence anisotropy	490 nm excitation; 530 nm emission filter	490 nm excitation; 530 nm emission filter	483 nm excitation; 536 nm emission filter
Program or method used for calculating data	Nonlinear least squares regression, Prism, Graphpad (San Diego, CA)	Nonlinear binding isotherm	Nonlinear least squares regression, Prism, Graphpad (San Diego, CA)
Data plotted as	Percent inhibition vs. competitor conc.	Percent inhibition vs. competitor conc.	Millipolarization vs. conc. of chemicals
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	IC_{50} E_2/IC_{50} ligand X100	IC_{50} E_2/IC_{50} ligand X100	IC_{50} E_2/IC_{50} ligand X100

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity

Assays Using Fluorescent Polarization

Reference	Saito et al. (2000)
Preparation of receptor	
Species and subtype of receptor	human ERα
Source of receptor	PanVera
Whole, truncated, recombinant, or chimeric	recombinant
Buffer for assay of receptor	40 mM Tris-HCL, pH 7.5, 50 mM KCL, 5% glycerol, 10% dimethylformamide, 0.02% Na azide, 50 µg/mL bovine gamma globulin
Protein concentration	n.p.
Competitive binding assay	
Ligand used	ES2
Concentration of estrogen	1 nM
Fluorescent ligand	FES1 ERα 13 nM, ERβ 10 nM
Concentration of fluorescent ligand	2 nM
Solvent used to dissolve competing ligand	10 mM ethanol
Concentration range of competing ligand	10 nM -10 µM
Number of replicates	3
Number of times assay repeated	n.p.
Time of incubation	60 min
Temperature of incubation	room temp
Data calculations	
Fluorescence anisotropy	490 nm excitation; 530 nm emission filter
Program or method used for calculating data	n.p.
Data plotted as	Percent inhibition vs. competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	n.p.
Calculation of RBA	n.p.

Abbreviations: n.p. = not provided; n.a. = not applicable; RBA = relative binding affinity