Assays Using Human ERα and ERβ

Reference	Arcaro et al. (1999)	Arcaro et al. (2000)	Fertuck et al. (2001)
Preparation of receptor			
Species and subtype of receptor	human ER alpha and human ER beta	human ER alpha	human ER beta
Source of receptor	n.p.	n.p.	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	recombinant
Buffer for isolation of receptor	n.a.	n.a.	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DDT, 10% glycerol containing 1 mg/mL BSA pH 7.6)
Protein concentration	1.2 nM	1.2 nM	n.p.
Competitive binding assay			
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	2.5 nM	2.5 nM	10 pM - 1 μM
Solvent used to dissolve ligand	n.p.	n.p.	DMSO
Concentration range of competing ligand	5 nM - 100 μM	0.1 μM - 10 μM	60 nM - 20μM
Number of replicates	3	3	4
Number of times assay repeated	3	2	3
Time of incubation	4 hours	4 hours	24 hours
Temperature of incubation	room temperature	room temperature	4°C
Nonspecific binding measured (y/n)	y	y	n.p.
Separation of ligand			
Type of slurry	hydroxyapatite	hydroxyapatite	n.p.
Incubation time and temperature	15 min; n.p.	15 min; n.p.	n.p.
Centrifugation time and temperature	20 min; n.p.	20 min; n.p.	n.p.
Data calculations			
Program or method used for calculating data	SigmaPlot	SigmaPlot	Nonlinear regression using Graphpad Prism 3.0
Data plotted as	% ^{3}H-E_2 bound vs. log M of ligand	% ^{3}H-E_2 bound vs. log M of ligand	Specific binding vs. log competitor conc.
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	n.p.	n.p.	IC_{50} E_2/IC_{50} competitor

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

Assays Using Human ERα and ERβ

Reference	Gaido et al. (1999)	Klotz et al. (1996)	Kraichely et al. (2000)
Preparation of receptor			
Species and subtype of receptor	human ER alpha and human ER beta	human ER alpha	human ER alpha and human ER beta
Source of receptor	PanVera	Produced in Sf9 insect cells using a baculovirus expression system	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant	recombinant
Buffer for isolation of receptor	n.a.	n.p.	n.a.
Protein concentration	8 pmol/mL (alpha) 11 pmol/mL (beta)	0.4 nM	1.5 nM
Competitive binding assay			
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	5 nM	2.5 nM	10 nM
Solvent used to dissolve ligand	n.p.	dimethyl sulfoxide or ethanol	n.p.
Concentration range of competing ligand	0.1 nM - 10 µM	10 nM - 100 µM	n.p.
Number of replicates	3	3	n.p.
Number of times assay repeated	3	2	n.p.
Time of incubation	overnight	1 hour	18 hours
Temperature of incubation	4ºC	25ºC	0ºC
Nonspecific binding measured (y/n)	n.p.	y	n.p.
Separation of ligand			
Type of slurry	hydroxyapatite	5% activated charcoal/0.5% dextran	hydroxyapatite
Incubation time and temperature	30 min; 4ºC	10 min; 4ºC	15 min; 0ºC
Centrifugation time and temperature	10 min; n.p.	3 min; n.p.	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
Program or method used for calculating data	GraphPad Prism software	n.p.	n.p.
Data plotted as	% Binding vs. log dose (M)	% ^{3}H-E_2 bound vs. [ligand] in nM	no plot of data reported
Data format in paper (*e.g.,* IC_{50}, K_i)	RBA	IC_{50}	RBA
Calculation of RBA	IC_{50} E_2/IC_{50} competitor	n.p.	n.p.

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

Assays Using Human ERα and ERβ

Reference	Meyers et al. (1999)	Sun et al. (1999)	Sun et al. (1999)
Preparation of receptor			
Species and subtype of receptor	human ER alpha and human ER beta	human ER alpha ligand binding domain	human ER beta ligand binding domain
Source of receptor	PanVera	expressed in E coli using pET15b vector	expressed in E coli using pET15b vector
Whole, truncated, recombinant, or chimeric	recombinant	truncated (amino acids 304 - 554)	truncated (amino acids 256 - 505)
Buffer for isolation of receptor	n.a.	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol	50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM butylated hydroxyanisole, 10 mM mercaptoethanol
Protein concentration	1.5 nM	n.p.	n.p.
Competitive binding assay			
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	10 nM	10 nM	10 nM
Solvent used to dissolve ligand	n.p.	n.p.	n.p.
Concentration range of competing ligand	n.p.	n.p.	n.p.
Number of replicates	2	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	18 - 24 hours	18 hours	18 hours
Temperature of incubation	0ºC	0ºC	0ºC
Nonspecific binding measured (y/n)	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry	hydroxyapatite	hydroxylapatite	hydroxylapatite
Incubation time and temperature	15 min; 0ºC	15 min; 0ºC	15 min; 0ºC
Centrifugation time and temperature	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer	Washed 3X with 1 mL of 0.05 M Tris, pH 7.3 buffer
Data calculations			
Program or method used for calculating data	n.p.	Ki calculated using Cheng-Prusoff equation	Ki calculated using Cheng-Prusoff equation
Data plotted as	no plot of data reported	no plot of data reported	no plot of data reported
Data format in paper (*e.g.,* IC_{50}, K_i)	RBA	IC_{50} (not reported), Ki, and RBA	IC_{50} (not reported), Ki, and RBA
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x100	IC_{50} E_2/IC_{50} competitor x100

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

Assays Using Human ERα and ERβ

Reference	Vakharia and Gierthy (1999)	Vakharia and Gierthy (2000)
Preparation of receptor		
Species and subtype of receptor	human ER alpha	human ER alpha
Source of receptor	PanVera	PanVera
Whole, truncated, recombinant, or chimeric	recombinant	recombinant
Buffer for isolation of receptor	n.a.	n.a.
Protein concentration	1.2 nM	1.25 nM
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	2.5 nM	2.5 nM
Solvent used to dissolve ligand	dimethyl sulfoxide	dimethyl sulfoxide
Concentration range of competing ligand	10 nM -1000 µM	50 nM - 50 µM
Number of replicates	3	3
Number of times assay repeated	n.p.	n.p.
Time of incubation	4 hours	4 hours
Temperature of incubation	room temperature	room temperature
Nonspecific binding measured (y/n)	y	y
Separation of ligand		
Type of slurry	hydroxyapatite	hydroxyapatite
Incubation time and temperature	n.p.	n.p.
Centrifugation time and temperature	10 min; n.p.	10 min; n.p.
Data calculations		
Program or method used for calculating data	Sigmaplot software	Sigmaplot software
Data plotted as	% ^{3}H-E_2 bound vs. [ligand] in nM	% ^{3}H-E_2 bound vs. [ligand] in nM
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	IC_{50}
Calculation of RBA	n.a.	n.a.

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