Assays Using Recombinant ERα and ERβ

Reference	Kuiper et al. (1997)	Kuiper et al. (1997)	Kuiper et al. (1998) [method a]
Preparation of receptor			
Species and subtype of receptor	rat ER beta	human ER alpha	human ER beta
Whole, truncated, recombinant, or chimeric	whole recombinant	whole recombinant	whole recombinant
Method of protein synthesis	*in vitro* using TnT-coupled reticulocyte lysate system	*in vitro* using TnT-coupled reticulocyte lysate system	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained
RNA polymerase	T7-RNA polymerase	T7-RNA polymerase	n.a.
Reaction time or cell growth time	90 min reaction time	90 min reaction time	48 hours cell growth time
Reaction temperature	30ºC	30ºC	n.a.
Buffer for dilution of translation mixture or nuclear extract	20 mM HEPES, pH 7.9; 150 mM NaCl, 10% w/v glycerol, 1 mM EDTA, 6 mM Na_2MoO_4	20 mM HEPES, pH 7.9; 150 mM NaCl, 10% w/v glycerol, 1 mM EDTA, 6 mM Na_2MoO_4	17 mM K_2HPO_4, 3 mM KH_2PO_4, 40 mM KCl, 6 mM monothioglycerol, pH=7.6
Protein concentration	10 - 15 pM	10 - 15 pM	800 pM
Competitive binding assay			
Radioligand used	16α-[^{125}I]-estradiol	16α-[^{125}I]-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	125 - 150 pM	125 - 150 pM	3 nM
Solvent used to dissolve ligand	dimethyl sulfoxide	dimethyl sulfoxide	dimethyl sulfoxide
Concentration range of competing ligand	0.001 - 100 µM	0.001 - 100 µM	n.p.
Volume of translation mixture or nuclear extract	2 µL	0.25 µL	200 µL nuclear extract per Scintistrip well
Time to allow adhesion of ER to Scintistrip wells	n.a.	n.a.	18 hours then washed 2X with buffer
Temperature to allow adhesion	n.a.	n.a.	ambient temperature
Number of replicates	2	2	n.p.
Number of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	16 hours	16 hours	18 hours
Temperature of incubation	4ºC	4ºC	ambient temperature
Nonspecific binding measured (y/n)	y	y	n.p.
Separation of ligand			
Type of column	Gel filtration over Sephadex G-25 column	Gel filtration over Sephadex G-25 column	Solid-phase ligand binding using Scintistrip wells
Data calculations			
Program or method used for calculating data	Nonlinear 4-parameter logistic model to estimate IC_{50} and Cheng-Prusoff equation to calculate Ki	Nonlinear 4-parameter logistic model to estimate IC_{50} and Cheng-Prusoff equation to calculate Ki	Nonlinear 4-parameter logistic model to estimate IC_{50}
Data plotted as	% [^{125}I]-E_2 bound vs. log M of compound	% [^{125}I]-E_2 bound vs. log M of compound	no plot of data reported
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50} (not reported), Ki and RBA	IC_{50} (not reported), Ki and RBA	IC_{50} (not reported) and RBA
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

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

Assays Using Recombinant ERα and ERβ

Reference	Kuiper et al. (1998) [method a]	Kuiper et al. (1998) [method b]
Preparation of receptor		
Species and subtype of receptor	human ER alpha	human ER beta
Whole, truncated, recombinant, or chimeric	whole recombinant	whole recombinant
Method of protein synthesis	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained
RNA polymerase	n.a.	n.a.
Reaction time or cell growth time	48 hours cell growth time	48 hours cell growth time
Reaction temperature	n.a.	n.a.
Buffer for dilution of translation mixture or nuclear extract	17 mM K_2HPO_4, 3 mM KH_2PO_4, 40 mM KCl, 6 mM monothioglycerol, pH=7.6	20 mM HEPES, pH 7.5; 150 mM KCl, 1 mM EDTA, 6mM monothioglycerol, 8.7% (v/v) glycerol
Protein concentration	400 pM	0.3 - 0.4 nM
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	3 nM	3 nM
Solvent used to dissolve ligand	dimethyl sulfoxide	dimethyl sulfoxide
Concentration range of competing ligand	n.p.	n.p.
Volume of translation mixture or nuclear extract	200 µL nuclear extract per Scintistrip well	n.p.
Time to allow adhesion of ER to Scintistrip wells	18 hours then washed 2X with buffer	n.a.
Temperature to allow adhesion	ambient temperature	n.a.
Number of replicates	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.
Time of incubation	18 hours	18 - 20 hours
Temperature of incubation	ambient temperature	6°C
Nonspecific binding measured (y/n)	n.p.	n.p.
Separation of ligand		
Type of column	Solid-phase ligand binding using Scintistrip wells	Gel filtration over Sephadex G-25 column
Data calculations		
Program or method used for calculating data	Nonlinear 4-parameter logistic model to estimate IC_{50}	Nonlinear 4-parameter logistic model to estimate IC_{50}
Data plotted as	no plot of data reported	dpm bound radioligand vs. log M of compound
Data format in paper (e.g., IC_{50}, K_i)	IC_{50} (not reported) and RBA	IC_{50} (not reported) and RBA
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

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

Assays Using Recombinant ERα and ERβ

Reference	Kuiper et al. (1998) [method b]	Morito et al. (2001)
Preparation of receptor		
Species and subtype of receptor	human ER alpha	human ER alpha; human ER beta
Whole, truncated, recombinant, or chimeric	whole recombinant	whole recombinant
Method of protein synthesis	Sf9 cells were infected with amplified baculovirus; infected cells were harvested after 48 h, and a nuclear fraction containing ER beta was obtained	Sf9 cells were infected with amplified baculovirus; harvested after 72 h and a cytosolic fraction made by sonication and centrifugation of the homogenate containing the ER alpha or ER beta
RNA polymerase	n.a.	n.a.
Reaction time or cell growth time	48 hours cell growth time	72 hours growth of cells
Reaction temperature	n.a.	28°C
Buffer for dilution of translation mixture or nuclear extract	20 mM HEPES, pH 7.5; 150 mM KCl, 1 mM EDTA, 6mM monothioglycerol, 8.7% (v/v) glycerol	40 mM Tris-HCL, pH 7.4, 0.5mM EDTA, 0.2M KCL, 10% (v/v) glycerol,1mM dithiothreitol, 1mM PMSF
Protein concentration	0.3 - 0.4 nM	36 µg/mL
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Concentration of radioligand	3 nM	2.5 pmoles
Solvent used to dissolve ligand	dimethyl sulfoxide	n.p.
Concentration range of competing ligand	n.p.	n.p.
Volume of translation mixture or nuclear extract	n.p.	5 µL
Time to allow adhesion of ER to Scintistrip wells	n.a.	n.a.
Temperature to allow adhesion	n.a.	n.a.
Number of replicates	n.p.	n.p.
Number of times assay repeated	n.p.	n.p.
Time of incubation	18 - 20 hours	16 hours
Temperature of incubation	6°C	0°C
Nonspecific binding measured (y/n)	n.p.	n.p.
Separation of ligand		
Type of column	Gel filtration over Sephadex G-25 column	0.5% activated charcoal and 0.05% dextran
Data calculations		
Program or method used for calculating data	Nonlinear 4-parameter logistic model to estimate IC_{50}	n.p.
Data plotted as	dpm bound radioligand vs. log M of compound	%^{3}H E_2 bound vs. fold excess of estradiol
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50} (not reported) and RBA	Calculated IC_{50} by knowing that 1 fold increase was 5nM
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100

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