Assays Using GST-ERdef Constructs

Reference	Fertuck et al. (2001)	Matthews and Zacharewski (2001)	Matthews et al. (2000)
Preparation of receptor			
Species and subtype of receptor	GST-hERα	GST-hERαdef, -aERdef, -cERdef, -rtERdef	GST-hERαdef, -aERdef, -cERdef, -rtERdef
Whole, truncated, recombinant, or chimeric	Recombinant, truncated	Recombinant, truncated fusion protein	Recombinant, truncated fusion protein
cDNA contained in	pGEX-hERαdef	pGEX-ERdef	pGEX-ERdef
Buffer for dilution of receptor	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 1 mM DTT, 10% glycerol containing 1mg/mL BSA, pH 7.6)
Protein concentration	1 mg/mL	1 mg/mL	1 mg/mL
Competitive binding assay			
Radioligand used and volume	5 µL of ^{3}H-E_2	5 µL of ^{3}H-E_2	5 µL of ^{3}H-E_2
Concentration of radioligand	2.5 nM	0.1 - 3.5 nM	0.1 - 3.5 nM
Solvent used to dissolve ligand	DMSO	5 µL DMSO	5 µL DMSO
Concentration range of competing ligand	60 nM - 20 µM	1 nM - 10 µM	1 nM - 10 µM
Volume of receptor	240 µL	240 µL	240 µL
Number of replicates	4	4	4
Number of times assay repeated	3	n.p.	n.p.
Time of incubation	2 hours	2 hours	2 hours
Temperature of incubation	4ºC	4ºC	4ºC
Nonspecific binding measured (y/n)	n.p.	y, 400x excess E_2	y, 400x excess E_2
Separation of ligand			
Type of column	n.p.	96-well filter plate and harvester of bound radioligand	96-well filter plate and harvester of bound radioligand
Washing solution	n.p.	TEG buffer (10mMTris, pH 7.6 1.5mM EDTA, 1mM DDT, 10% glycerol containing 1mg/mL BSA)	TEG buffer (10mMTris, pH 7.6 1.5mM EDTA, 1mM DDT, 10% glycerol containing 1mg/mL BSA)
Data calculations			
Program or method used for calculating data	Nonlinear regression	Nonlinear regression using Graphpad Prism 3.0	Nonlinear regression using Graphpad Prism 3.0
Data plotted as	Specific binding vs. log competitor conc.	Percent specific binding vs. log competitor conc.	Percent 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	IC_{50} E_2/IC_{50} ligand	IC_{50} E_2/IC_{50} ligand	IC_{50} E_2/IC_{50} ligand

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