Assays Using MCF-7 Cells and Cytosol

Reference	Arcaro et al. (1999)	Brooks et al. (1987)	Brooks et al. (1987)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	whole cells	homogenate	whole cells
Serum source	5% calf serum	10% calf serum	n.p.
Serum stripping method	n.p.	n.p.	n.p.
Residual E_2 in serum	n.p.	n.p.	n.p.
No. of treated cells	n.p.	n.p.	n.p.
Buffer for preparation of cell homogenate or cytosol	n.p.	Tris-EDTA + reducing agent, pH 7.4	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	0.1 nM	n.p.	3 nM
Specific activity of labelled E_2	140 - 150 Ci/mmol	n.p.	n.p.
Test chemical solvent	DMSO	n.p.	n.p.
Concentration range of competing ligand	5, 1, 0.5 µM	n.p.	n.p.
No. of replicates	quadruplicate	n.p.	n.p.
Time of incubation	3 hours	n.p.	1 hour
Temperature of incubation	37ºC	n.p.	37ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	ethanol in PBS	charcoal/dextran in Tris-EDTA, pH 7.4	ethanol
Incubation time and temperature	n.p.	n.p.	n.p.
Centrifugation speed	n.p.	1000g	n.p.
Centrifugation time and temperature	n.p.	10 min, 4ºC	n.p.
Resuspension volume and buffer for pellet	200 µL	n.p.	n.p.
Extraction of label	ethanol	n.p.	ethanol
Data calculations			
Program or method used to calculate data	SigmaPlot	n.p.	n.p.
Data plotted as	linear regression	n.p.	n.p.
Data format in paper (e.g., IC_{50}, K_i)	% displacement of E_2	n.p.	n.p.
Calculation of RBA	from IC_{50}	Scatchard plot	Scatchard plot

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

Assays Using MCF-7 Cells and Cytosol

Reference	Dodge et al. (1996)	Kramer et al. (1997)	Lascombe et al. (2000)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	cell lysate	cytosol	whole cells
Serum source	10% fetal bovine serum	10% fetal bovine serum	0.1% bovine serum
Serum stripping method	dextran/charcoal	dextran/charcoal	dextran/charcoal
Residual E_2 in serum	n.p.	5 pg/ml (18 pm)	n.p.
No. of treated cells	n.p.	n.p.	monolayer culture
Buffer for preparation of cell homogenate or cytosol	n.p.	Tris-EDTA-DTT-molybdate, pH 7.5; 4ºC	n.p.
Protein concentration of cytosol	0.5 mg/ml	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	0.5 nM	10 nM	0.1 nM
Specific activity of labelled E_2	n.p.	n.p.	n.p.
Test chemical solvent	n.p.	ethanol	ethanol
Concentration range of competing ligand	0.00001 - 1 µM	70 - 0.01 µM	n.p.
No. of replicates	n.p.	duplicate	quadruplicate
Time of incubation	18 hours	2 hours	1 hour
Temperature of incubation	4ºC	4ºC	37ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	charcoal/dextran; 0.07 ml	hydroxyapatite	ethanol in phosphate buffer, pH 7.4
Incubation time and temperature	n.p.	n.p.	n.p.
Centrifugation speed	n.p.	800g	n.p.
Centrifugation time and temperature	n.p.	10 min, 4ºC	n.p.
Resuspension volume and buffer for pellet	n.p.	n.p.	n.p.
Extraction of label	n.p.	n.p.	ethanol
Data calculations			
Program or method used to calculate data	n.p.	n.p.	Student's t-test
Data plotted as	n.p.	nonlinear regression	% control vs. molar excess of competitor
Data format in paper (e.g., IC_{50}, K_i)	DPM/nM	log IC_{50}	bound E_2 vs molar excess
Calculation of RBA	from IC_{50}	Scatchard plot	% control

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

Assays Using MCF-7 Cells and Cytosol

Reference	Miodini et al. (1999)	Nagel et al. (1997)	Palomino et al. (1994)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	homogenate	whole cells	cytosol
Serum source	2% fetal calf	calf serum	n.p.
Serum stripping method	n.p.	charcoal	n.p.
Residual E_2 in serum	n.p.	n.p.	n.p.
No. of treated cells	n.p.	n.p.	n.p.
Buffer for preparation of cell homogenate or cytosol	K_2HPO_4-EDTA, glycerol, thioglycerol, pH 7.4	n.p.	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	5 nM (16α-I-estradiol)	1 nM	1.5 nM
Specific activity of labelled E_2	8150 GBq/mM	104 Ci/mol	100 Ci/mmol
Test chemical solvent	n.p.	ethanol	n.p.
Concentration range of competing ligand	0.0025 - 25 µM	0.1 - 100 µM	1.5 - 3,000 nM
No. of replicates	quadruplicate	n.p.	triplicate
Time of incubation	overnight	18 hours	overnight
Temperature of incubation	4ºC	37ºC	4ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	n.p.	HBSS/BSA	charcoal/dextran
Incubation time and temperature	n.p.	n.p.	n.p.
Centrifugation speed	n.p.	n.p.	n.p.
Centrifugation time and temperature	n.p.	n.p.	n.p.
Resuspension volume and buffer for pellet	n.p.	1 mL	n.p.
Extraction of label	n.p.	HBSS	n.p.
Data calculations			
Program or method used to calculate data	Latin Square	n.p.	n.p.
Data plotted as	n.p.	n.p.	n.p.
Data format in paper (*e.g.*, IC_{50}, K_i)	% binding	% inhibition vs M	n.p.
Calculation of RBA	n.p.	RBA	Scatchard plot

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

Assays Using MCF-7 Cells and Cytosol

Reference	Rijks et al. (1996)	Soto et al. (1995)	Stoessel and Leclercq (1986)
Preparation of receptor			
Species or cell line	MCF-7 cells	MCF-7 cells	MCF-7 cells
Whole cells/cell homogenate	homogenate	cytosol	whole cells
Serum source	n.p.	plasma-derived human	fetal calf serum
Serum stripping method	n.p.	dextran/charcoal	
Residual E_2 in serum	n.p.	<0.01 pg/ml	
No. of treated cells		n.p.	monolayer culture
Buffer for preparation of cell homogenate or cytosol	Tris-EDTA-DTT-molybdate, pH 7.4; 4ºC	KCl-EDTA-Tris, pH 7.4	n.p.
Protein concentration of cytosol	1.4 mg/ml	n.p.	n.p.
Competitive binding assay			
Volume and concentration of ^{3}H-estradiol	$4.8x10^{-9}$	2 nM	1 nM
Specific activity of labelled E_2	4.26 TBq/mmol	n.p.	100 Ci/mmol
Test chemical solvent	n.p.	DMSO or ethanol	ethanol
Concentration range of competing ligand	$1x10^{-11}$ - $2x10^{-6}$	1 pM - 1 mM	0.1 nM - 10 µM
No. of replicates	duplicate	n.p.	triplicate
Time of incubation	18 hours	16 hours	50 min
Temperature of incubation	0 - 4ºC	4ºC	37ºC
Measure of nonspecific binding	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	charcoal/dextran in SHBG	charcoal/dextran	n.p.
Incubation time and temperature	4ºC	n.p.	n.p.
Centrifugation speed	800g	n.p.	n.p.
Centrifugation time and temperature	7 min; 4ºC	n.p.	n.p.
Resuspension volume and buffer for pellet	n.p.	n.p.	n.p.
Extraction of label	n.p.	n.p.	n.p.
Data calculations			
Program or method used to calculate data	n.p.	n.p.	n.p.
Data plotted as	n.p.	n.p.	n.p.
Data format in paper (e.g., IC_{50}, K_i)	n.p.	n.p.	RBA
Calculation of RBA	n.p.	n.p.	IC_{50} E_2/IC_{50} test compound X 100

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

Assays Using MCF-7 Cells and Cytosol

Reference	VanderKuur et al. (1993)
Preparation of receptor	
Species or cell line	MCF-7 cells
Whole cells/cell homogenate	whole cells
Serum source	5% calf serum
Serum stripping method	none
Residual E_2 in serum	none
No. of treated cells	n.p.
Buffer for preparation of cell homogenate or cytosol	n.p.
Protein concentration of cytosol	n.p.
Competitive binding assay	
Volume and concentration of ^{3}H-estradiol	n.p.
Specific activity of labelled E_2	n.p.
Test chemical solvent	n.p.
Concentration range of competing ligand	n.p.
No. of replicates	n.p.
Time of incubation	n.p.
Temperature of incubation	4ºC
Measure of nonspecific binding	n.p.
Separation of ligand	
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	charcoal/dextran
Incubation time and temperature	n.p.
Centrifugation speed	n.p.
Centrifugation time and temperature	n.p.
Resuspension volume and buffer for pellet	n.p.
Extraction of label	n.p.
Data calculations	
Program or method used to calculate data	n.p.
Data plotted as	n.p.
Data format in paper (e.g., IC_{50}, K_i)	n.p.
Calculation of RBA	Scatchard plot

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