Assays Using Mouse Uterine Cytosol

Reference	Chae et al. (1991)	Connor et al. (1997)	Fielden et al. (1997)	Korach et al. (1978)
Preparation of receptor				
Species or cell line from which receptor obtained	CD-1 (ICR) BR mice	B6C3F1 mice	CD-1 mice	CD-1 mice
Age of animals/cells	8-10 weeks	24 days	Shortly after weaning	26 days
Source of receptor	Uterus	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl2, pH 7.6)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thioglycerol, 10mM sodium molybdate)	Ice cold TEGD buffer (10 mM tris base, 1.5 mM EDTA, 10% glycerol, 1 mM dithiothreitol, pH 7.6)	Ice cold Tris/EDTA/ glycerol
Dilution of tissue with buffer	50 mg tissue/mL buffer	50 mg tissue/mL buffer	50 mg tissue/ mL buffer	n.p.
Protein concentration of cytosol	n.p.	n.p.	2.0 mg/mL	2 mg/mL
Ammonium sulfate fractionation	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	vol n.p.; final conc 5 nM	vol n.p.; final conc 10 nM	30 µL; final conc 1 nM	vol n.p.; final conc 0.1 nM
Specific activity of radioligand	n.p.	130 Ci/mmol	130 Ci/mmol	110 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	n.p.	dimethyl sulfoxide	n.p.
Concentration range of competing ligand	0.5 nM - 5 µM	n.p.	1 nM - 1000 µM	n.p.
Volume of ER prep used	100 µL	n.p.	240 µL	100 µL
No. of replicates	2	1	2	n.p.
No. of times assay repeated	3	3	n.p.	n.p.
Incubation time and temperature	18 hours; 4º C	8 hours; 4º C	30º C for 30 min; then cooled to 4º C	18 hours; 0-4º C
Measured nonspecific binding (y/n)	y	n.p.	y	n.p.
Separation of ligand				
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µl hydroxyapatite in TEGM	0.1 vol dextran-coated charcoal	125 µl 60% (v/v) hydroxylapatite suspension in TEGD buffer	Protamine sulfate
Incubation time and temperature	n.p.	On ice	n.p.	10 min/4ºC
Centrifugation speed	1,000 g	8,000 g	n.p.	2,000 g
Centrifugation time and temperature	10 min; temp n.p.	10 min; temp n.p.	n.p.	10 min/4ºC
Data calculations				
Program or method used for calculating data	Ligand Competition Analysis Software by EMF	n.p.		n.p.
Data plotted as	% receptor bound vs. molar excess competitor	No plot	Percent specific binding of [^{3}H]E_2 vs. log concentration of competitor (M)	n.p.
Data format in paper (e.g., IC_{50}, K_i)	C_{50} and RBA	IC_{50}	IC_{50}	RBA
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x100	n.p.	IC_{50} E_2/IC_{50} competitor x100

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

Assays Using Mouse Uterine Cytosol

Reference	Korach et al. (1979)	Korach et al. (1985)	Korach et al. (1988)	Korach et al. (1989)
Preparation of receptor				
Species or cell line from which receptor obtained	CD-1 mice	CD-1 (ICR) BR mice	CD-1 mice	CD-1 (ICR) BR mice
Age of animals/cells	On or before 24 days	n.p.	On or before 24 days	n.p.
Source of receptor	Uterus	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol	Cytosol
When ovariectomized	5 days prior to sacrifice	7 days prior to sacrifice	5 days prior to sacrifice	7 days prior to sacrifice
Buffer for preparation of cytosol	Ice cold TEG buffer (10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, pH 8.0)	Ice cold TEGM buffer (pH 8.0, 4ºC, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl2)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)	Ice cold TEGM buffer (pH 8.0, 4ºC, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl2)
Dilution of tissue with buffer	n.p.	n.p.	75 mg wet weight/mL buffer	50 mg tissue/mL buffer
Protein concentration of cytosol	1 mg/mL approximate	1 mg/mL approximate	n.p.	1.7 mg/mL
Ammonium sulfate fractionation	n.a.	n.a.	Cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation	n.a.
Competitive binding assay				
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	vol n.p.; final conc 5 nM	vol n.p.; final conc 5 nM	n.p.	vol n.p.; final conc 5 nM
Specific activity of radioligand	110 Ci/mmol	98 Ci/mmol	n.p.	98 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	n.p.	n.p.	n.p.
Concentration range of competing ligand	1 nM - 2.5 µM	1 nM - 2.5 µM	n.p.	0.5 - 500 mM
Volume of ER prep used	100 µL	100 µL	n.p.	200 µL
No. of replicates	3	n.p.	n.p.	
No. of times assay repeated	5 or more	n.p.	n.p.	4
Incubation time and temperature	18 hours; 4º C	18 hours; 4º C	n.p.	18 hours; 4º C
Measured nonspecific binding (y/n)	n.p.	n.p.	y	n.p.
Separation of ligand				
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Protamine sulfate	Protamine sulfate	Hydroxylapatite adsorption	Hydroxylapatite adsorption
Incubation time and temperature	10 min/4ºC	10 min/4ºC	10 min/4ºC	n.p.
Centrifugation speed	2,000 g	2,000 g	2,000 g	n.p.
Centrifugation time and temperature	10 min/4ºC	10 min/4ºC	10 min/4ºC	n.p.
Data calculations				
Program or method used for calculating data	n.p.	n.p.	n.p.	n.p.
Data plotted as	Semi-log plot of % Receptor bound vs. log molar excess of unlabeled competitor	Scatchard plot (bound/unbound vs. bound)	Semi-log plot of % Receptor bound vs. log molar excess of unlabeled competitor	% receptor bound vs. molar excess competitor
Data format in paper (e.g., IC_{50}, K_i)	IC_{50}	C_{50} (molar equivalents of unlabeled competitor required to occupy 50% of the receptor binding sites)	C_{50}	C_{50}
Calculation of RBA	n.p.	n.p.	n.p.	n.p.

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

Assays Using Mouse Uterine Cytosol

Reference	Korach (1979)	Matthews et al. (2001)	Ramamoorthy et al. (1997a)	Ramamoorthy et al. (1997b)
Preparation of receptor				
Species or cell line from which receptor obtained	CD-1 mice	CD-1 mice	B6C3F1 mice	B6C3F1 mice
Age of animals/cells	24 days	21 days	24 days	24 days
Source of receptor	Uterus	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol	Cytosol
When ovariectomized	5 days prior to sacrifice	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	Ice cold TE buffer(0.01 M Tris, .0015 M disodium EDTA, pH 8 or 7.4)	TEGD (10 nM Tris base, 1.5 mM EDTA, 10% glycerol, 1.0 mM DTT, pH 7.6)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)	Ice cold TESHMo buffer (10 mM Tris-Cl, pH 7.4, 1.5 mM EDTA, 15 mM thio-glycerol, 10mM sodium molybdate)
Dilution of tissue with buffer	n.p.	50 mg tissue/ mL buffer	50 mg tissue/mL buffer	50 mg tissue/mL buffer
Protein concentration of cytosol	1 mg/mL approximate	2.0 mg/mL	n.p.	n.p.
Ammonium sulfate fractionation	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	vol n.p.; final conc 10 nM	5 µL; final conc 2.5 nM	n.p.	vol n.p.; final conc 10 nM
Specific activity of radioligand	110 Ci/mmol	n.p.	130 Ci/mmol	n.p.
Solvent used to dissolve competing ligand	n.p.	dimethyl sulfoxide	n.p.	n.p.
Concentration range of competing ligand	0.1 - 1000-fold molar excess	n.p.	10 nM - 10 µM	n.p.
Volume of ER prep used	100 µL	240 µL	n.p.	n.p.
No. of replicates	n.p.	4	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.	n.p.
Incubation time and temperature	18 hours; 4º C	2 hours; 30º C	8 hours; 4º C	16-18 hours; 4º C
Measured nonspecific binding (y/n)	n.p.	n.p.	n.p.	n.p.
Separation of ligand				
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Protamine sulfate	96-well filter plate and vacuum pump harvester	0.1 vol DCC suspension (0.5% dextran: 5% charcoal, wt/vol in TESHMo)	0.1 vol DCC suspension (0.5% dextran: 5% charcoal, wt/vol in TESHMo)
Incubation time and temperature	10 min/4ºC	Samples dried under suction for 30 sec; Filter plates sealed. Scintillation cocktail added to each well.	10 min; temp n.p.	10 min; temp n.p.
Centrifugation speed	n.p.	n.a.	5,000 g	5,000 g
Centrifugation time and temperature	10 min/4ºC	n.a.	10 min; temp n.p.	10 min; temp n.p.
Data calculations				
Program or method used for calculating data	n.p.	GraphPad Prism 3.0 software	n.p.	n.p.
Data plotted as	Scatchard plot	Percent specific binding of [^{3}H]E_2 vs. log competitor concentration	%[^{3}H]E_2 bound vs. log [M]	DPM vs. log [M]
Data format in paper (*e.g.,* IC_{50}, K_i)	C_{50} (molar excess of unlabeled competitor which inhibits 50% specific receptor binding)	IC_{50}	n.p.	IC_{50}
Calculation of RBA	n.p.	n.p.	n.p.	n.p.

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

Assays Using Mouse Uterine Cytosol

Reference	Shelby et al. (1996)	Waller et al. (1996)
Preparation of receptor		
Species or cell line from which receptor obtained	CD-1 BR mice	CD-1 mice
Age of animals/cells	Either 8-10 weeks or 12-14 weeks	On or before 24 days
Source of receptor	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol
When ovariectomized	2 weeks prior to sacrifice	5 days prior to sacrifice
Buffer for preparation of cytosol	TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl2, pH 7.6)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)
Dilution of tissue with buffer	50 mg tissue/mL buffer	75 mg wet weight/mL buffer
Protein concentration of cytosol	n.p.	n.p.
Ammonium sulfate fractionation	n.a.	Cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation for some binding experiments
Competitive binding assay		
Radioligand used	^{3}H-17β-estradiol	^{3}H-17β-estradiol
Volume, concentration of radioligand	final conc 5 nM	final conc 5 nM
Specific activity of radioligand	n.p.	n.p.
Solvent used to dissolve competing ligand	n.p.	n.p.
Concentration range of competing ligand	0.5 nM - 5 µM	2.5 nM - 25 µM
Volume of ER prep used	100 µL	100 µL
No. of replicates	2	n.p.
No. of times assay repeated	2	n.p.
Incubation time and temperature	18 hours; 4º C	18 hours; 4º C
Measured nonspecific binding (y/n)	n.p.	n.p.
Separation of ligand		
Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% hydroxyapatite in TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl2, pH 7.6)	Hydroxylapatite adsorption
Incubation time and temperature	n.p.	10 min/4ºC
Centrifugation speed	1,000 g	2,000 g
Centrifugation time and temperature	10 min; temp n.p.	10 min/4ºC
Data calculations		
Program or method used for calculating data	n.p.	n.p.
Data plotted as	Semi-log plot of % Receptor bound vs. Log competitor concentration (M)	n.p.
Data format in paper (*e.g.,* IC_{50}, K_i)	n.p.	n.p.
Calculation of RBA	n.p.	n.p.

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