

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				
<i>Species or cell line from which receptor obtained</i>	CD-1 (ICR) BR mice	B6C3F1 mice	CD-1 mice	CD-1 mice
<i>Age of animals/cells</i>	8-10 weeks	24 days	Shortly after weaning	26 days
<i>Source of receptor</i>	Uterus	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	Cytosol	Cytosol	Cytosol	Cytosol
<i>When ovariectomized</i>	n.p.	n.p.	n.p.	n.p.
<i>Buffer for preparation of cytosol</i>	TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl ₂ , 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
<i>Dilution of tissue with buffer</i>	50 mg tissue/mL buffer	50 mg tissue/mL buffer	50 mg tissue/ mL buffer	n.p.
<i>Protein concentration of cytosol</i>	n.p.	n.p.	2.0 mg/mL	2 mg/mL
<i>Ammonium sulfate fractionation</i>	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	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
<i>Specific activity of radioligand</i>	n.p.	130 Ci/mmol	130 Ci/mmol	110 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	n.p.	dimethyl sulfoxide	n.p.
<i>Concentration range of competing ligand</i>	0.5 nM - 5 µM	n.p.	1 nM - 1000 µM	n.p.
<i>Volume of ER prep used</i>	100 µL	n.p.	240 µL	100 µL
<i>No. of replicates</i>	2	1	2	n.p.
<i>No. of times assay repeated</i>	3	3	n.p.	n.p.
<i>Incubation time and temperature</i>	18 hours; 4° C	8 hours; 4° C	30° C for 30 min; then cooled to 4° C	18 hours; 0-4° C
<i>Measured nonspecific binding (y/n)</i>	y	n.p.	y	n.p.
Separation of ligand				
<i>Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)</i>	250 µl hydroxyapatite in TEGM	0.1 vol dextran-coated charcoal	125 µl 60% (v/v) hydroxyapatite suspension in TEGD buffer	Protamine sulfate
<i>Incubation time and temperature</i>	n.p.	On ice	n.p.	10 min/4°C
<i>Centrifugation speed</i>	1,000 g	8,000 g	n.p.	2,000 g
<i>Centrifugation time and temperature</i>	10 min; temp n.p.	10 min; temp n.p.	n.p.	10 min/4°C
Data calculations				
<i>Program or method used for calculating data</i>	Ligand Competition Analysis Software by EMF	n.p.		n.p.
<i>Data plotted as</i>	% receptor bound vs. molar excess competitor	No plot	Percent specific binding of [³ H]E ₂ vs. log concentration of competitor (M)	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	C ₅₀ and RBA	IC ₅₀	IC ₅₀	RBA
<i>Calculation of RBA</i>	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				
<i>Species or cell line from which receptor obtained</i>	CD-1 mice	CD-1 (ICR) BR mice	CD-1 mice	CD-1 (ICR) BR mice
<i>Age of animals/cells</i>	On or before 24 days	n.p.	On or before 24 days	n.p.
<i>Source of receptor</i>	Uterus	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	Cytosol	Cytosol	Cytosol	Cytosol
<i>When ovariectomized</i>	5 days prior to sacrifice	7 days prior to sacrifice	5 days prior to sacrifice	7 days prior to sacrifice
<i>Buffer for preparation of cytosol</i>	Ice cold TEG buffer (10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, pH 8.0)	Ice cold TEGM buffer (pH 8.0, 4°C, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl ₂)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)	Ice cold TEGM buffer (pH 8.0, 4°C, 10 mM Tris, 1.5 mM disodium EDTA, 10% glycerol, 3mM MgCl ₂)
<i>Dilution of tissue with buffer</i>	n.p.	n.p.	75 mg wet weight/mL buffer	50 mg tissue/mL buffer
<i>Protein concentration of cytosol</i>	1 mg/mL approximate	1 mg/mL approximate	n.p.	1.7 mg/mL
<i>Ammonium sulfate fractionation</i>	n.a.	n.a.	Cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation	n.a.
Competitive binding assay				
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	vol n.p.; final conc 5 nM	vol n.p.; final conc 5 nM	n.p.	vol n.p.; final conc 5 nM
<i>Specific activity of radioligand</i>	110 Ci/mmol	98 Ci/mmol	n.p.	98 Ci/mmol
<i>Solvent used to dissolve competing ligand</i>	n.p.	n.p.	n.p.	n.p.
<i>Concentration range of competing ligand</i>	1 nM - 2.5 μM	1 nM - 2.5 μM	n.p.	0.5 - 500 mM
<i>Volume of ER prep used</i>	100 μL	100 μL	n.p.	200 μL
<i>No. of replicates</i>	3	n.p.	n.p.	
<i>No. of times assay repeated</i>	5 or more	n.p.	n.p.	4
<i>Incubation time and temperature</i>	18 hours; 4° C	18 hours; 4° C	n.p.	18 hours; 4° C
<i>Measured nonspecific binding (y/n)</i>	n.p.	n.p.	y	n.p.
Separation of ligand				
<i>Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)</i>	Protamine sulfate	Protamine sulfate	Hydroxylapatite adsorption	Hydroxylapatite adsorption
<i>Incubation time and temperature</i>	10 min/4°C	10 min/4°C	10 min/4°C	n.p.
<i>Centrifugation speed</i>	2,000 g	2,000 g	2,000 g	n.p.
<i>Centrifugation time and temperature</i>	10 min/4°C	10 min/4°C	10 min/4°C	n.p.
Data calculations				
<i>Program or method used for calculating data</i>	n.p.	n.p.	n.p.	n.p.
<i>Data plotted as</i>	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
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	IC ₅₀	C ₅₀ (molar equivalents of unlabeled competitor required to occupy 50% of the receptor binding sites)	C ₅₀	C ₅₀
<i>Calculation of RBA</i>	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				
<i>Species or cell line from which receptor obtained</i>	CD-1 mice	CD-1 mice	B6C3F1 mice	B6C3F1 mice
<i>Age of animals/cells</i>	24 days	21 days	24 days	24 days
<i>Source of receptor</i>	Uterus	Uterus	Uterus	Uterus
<i>Isolated preparation</i>	Cytosol	Cytosol	Cytosol	Cytosol
<i>When ovariectomized</i>	5 days prior to sacrifice	n.p.	n.p.	n.p.
<i>Buffer for preparation of cytosol</i>	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)
<i>Dilution of tissue with buffer</i>	n.p.	50 mg tissue/ mL buffer	50 mg tissue/mL buffer	50 mg tissue/mL buffer
<i>Protein concentration of cytosol</i>	1 mg/mL approximate	2.0 mg/mL	n.p.	n.p.
<i>Ammonium sulfate fractionation</i>	n.a.	n.a.	n.a.	n.a.
Competitive binding assay				
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	vol n.p.; final conc 10 nM	5 µL; final conc 2.5 nM	n.p.	vol n.p.; final conc 10 nM
<i>Specific activity of radioligand</i>	110 Ci/mmol	n.p.	130 Ci/mmol	n.p.
<i>Solvent used to dissolve competing ligand</i>	n.p.	dimethyl sulfoxide	n.p.	n.p.
<i>Concentration range of competing ligand</i>	0.1 - 1000-fold molar excess	n.p.	10 nM - 10 µM	n.p.
<i>Volume of ER prep used</i>	100 µL	240 µL	n.p.	n.p.
<i>No. of replicates</i>	n.p.	4	n.p.	n.p.
<i>No. of times assay repeated</i>	n.p.	n.p.	n.p.	n.p.
<i>Incubation time and temperature</i>	18 hours; 4° C	2 hours; 30° C	8 hours; 4° C	16-18 hours; 4° C
<i>Measured nonspecific binding (y/n)</i>	n.p.	n.p.	n.p.	n.p.
Separation of ligand				
<i>Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)</i>	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)
<i>Incubation time and temperature</i>	10 min/4°C	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.
<i>Centrifugation speed</i>	n.p.	n.a.	5,000 g	5,000 g
<i>Centrifugation time and temperature</i>	10 min/4°C	n.a.	10 min; temp n.p.	10 min; temp n.p.
Data calculations				
<i>Program or method used for calculating data</i>	n.p.	GraphPad Prism 3.0 software	n.p.	n.p.
<i>Data plotted as</i>	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]
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	C ₅₀ (molar excess of unlabeled competitor which inhibits 50% specific receptor binding)	IC ₅₀	n.p.	IC ₅₀
<i>Calculation of RBA</i>	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		
<i>Species or cell line from which receptor obtained</i>	CD-1 BR mice	CD-1 mice
<i>Age of animals/cells</i>	Either 8-10 weeks or 12-14 weeks	On or before 24 days
<i>Source of receptor</i>	Uterus	Uterus
<i>Isolated preparation</i>	Cytosol	Cytosol
<i>When ovariectomized</i>	2 weeks prior to sacrifice	5 days prior to sacrifice
<i>Buffer for preparation of cytosol</i>	TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl ₂ , pH 7.6)	TEG buffer (10 mM Tris, 1.5 mM EDTA, 10% glycerol, pH 7.6)
<i>Dilution of tissue with buffer</i>	50 mg tissue/mL buffer	75 mg wet weight/mL buffer
<i>Protein concentration of cytosol</i>	n.p.	n.p.
<i>Ammonium sulfate fractionation</i>	n.a.	Cytosolic ER was enriched by a 0-40% ammonium sulfate fractionation for some binding experiments
Competitive binding assay		
<i>Radioligand used</i>	³ H-17 -estradiol	³ H-17 -estradiol
<i>Volume, concentration of radioligand</i>	final conc 5 nM	final conc 5 nM
<i>Specific activity of radioligand</i>	n.p.	n.p.
<i>Solvent used to dissolve competing ligand</i>	n.p.	n.p.
<i>Concentration range of competing ligand</i>	0.5 nM - 5 μM	2.5 nM - 25 μM
<i>Volume of ER prep used</i>	100 μL	100 μL
<i>No. of replicates</i>	2	n.p.
<i>No. of times assay repeated</i>	2	n.p.
<i>Incubation time and temperature</i>	18 hours; 4° C	18 hours; 4° C
<i>Measured nonspecific binding (y/n)</i>	n.p.	n.p.
Separation of ligand		
<i>Volume and type of slurry (hydroxyapatite, charcoal, protamine sulfate)</i>	250 μL 60% hydroxyapatite in TEGM buffer (10 nM Tris, 1.5 mM EDTA, 10% glycerol, 3mM MgCl ₂ , pH 7.6)	Hydroxylapatite adsorption
<i>Incubation time and temperature</i>	n.p.	10 min/4°C
<i>Centrifugation speed</i>	1,000 g	2,000 g
<i>Centrifugation time and temperature</i>	10 min; temp n.p.	10 min/4°C
Data calculations		
<i>Program or method used for calculating data</i>	n.p.	n.p.
<i>Data plotted as</i>	Semi-log plot of % Receptor bound vs. Log competitor concentration (M)	n.p.
<i>Data format in paper (e.g., IC₅₀, K_i)</i>	n.p.	n.p.
<i>Calculation of RBA</i>	n.p.	n.p.

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