Assays Using Rat Uterine Cytosol

Reference	Acton et al. (1983)	Allen et al. (1980)	Anstead et al. (1989)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Rats (otherwise unspecified)	Rats (otherwise unspecified)
Age of animals	Mature	n.p.	n.p.
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	estradiol benzoate treated 3 x 0.16 µg	n.p.
Buffer for preparation of cytosol	n.p.	TED (10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol)	n.p.
Dilution of tissue with buffer	n.p.	8 uteri in 4 mL TED buffer	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 1 nM	50 µL; $7x10^{-9}$ mol/L	n.p.
Specific activity of radioligand	n.p.	58 Ci/mmol	n.p.
Solvent used to dissolve competing ligand	ethanol	TED buffer	n.p.
Concentration range of competing ligand	10^{-3} to 10 µg/mL	10^{-9} to $10^{-5.5}$ mol/L	10^{-3} to 10 µg/mL
Volume of ER prep used	n.p.	150 µL	n.p.
No. of replicates	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	16 hours	30 mins	n.p.
Temperature of incubation	3ºC	30ºC	n.p.
Measure of nonspecific binding(y/n) and concentration	y, 10^{-3} to 10 µg/mL	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Dextran-coated charcoal	200 µL dextran-coated charcoal (250mg% Norit A, 25mg% dextran in TED buffer)	Dextran-coated charcoal
Incubation time and temperature	n.p.	20 min. in ice cold water	n.p.
Centrifugation speed	n.p.	2000xg	n.p.
Centrifugation time and temperature	n.p.	4ºC for 5 min.	n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	n.p.	% specific ct./min. to controls vs. Conc. ligand in incubate (mol/L)	n.p.
Data format in paper (*e.g.,* IC_{50}, K_i)		graphical	n.p.
Calculation of RBA	IC_{50} E_2/IC_{50} ligand (estimated)	IC_{50} E_2/IC_{50} competitor x100	n.p.

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

Assays Using Rat Uterine Cytosol

Reference	Ashby et al. (1999)	Blair et al. (2000)	Connor et al. (1997)
Preparation of receptor			
Species/strain from which receptor obtained	AP Rats	Sprague Dawley rats	Sprague Dawley rats
Age of animals	21-25 days old	245±18 days old	24 days
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	10 days prior to sacrifice	n.p.
Buffer for preparation of cytosol	TEGM (10 mM Tris, 1.5 mM EDTA, $MgCl_2$ 3 mM, 10% glycerol, pH 7.6)	TEDG (10 mM Tris, 1.5 mM EDTA, 10 mM D, 10% glycerol, pH 7.4)	TESHMo (10 mM Tris-HCl, pH 7.4; 1.5 mM EDTA, 15 mM thioglycerol; 10 mM sodium molybdate)
Dilution of tissue with buffer	50 mg/mL buffer	100 mg/mL buffer	50 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 5 nM - 500 µM	10 µL; 1 nM	volume n.p.; 10 nM
Specific activity of radioligand	n.p.	141 Ci/mmol	130 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	100% ethanol	n.p.
Concentration range of competing ligand	5 nM - 500 µM	n.p.	1 mM - 0.1 µM
Volume of ER prep used	100 µL	50 µL	n.p.
No. of replicates	n.p.	2	3
No. of times assay repeated	n.p.	2	n.p.
Time of incubation	18 hours	20 hours	8 hours
Temperature of incubation	4ºC	4ºC	4ºC
Measure of nonspecific binding(y/n) and concentration	n.p.	y	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Hydroxyapatite in TEGM buffer	750 µl cold hydroxyapatite in 50mM Tris pH 7.4	0.1 volume Dextran-coated charcoal
Incubation time and temperature	n.p.	20 min. at 4ºC	n.p.
Centrifugation speed	n.p.	600xg	8000xg
Centrifugation time and temperature	n.p.	4ºC for 5 min.	10 min.; temp. n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	% Control vs. Concentration (M)	% [^{3}H]-E_2 bound vs. Competitor concentration (M)	n.p.
Data format in paper (e.g., IC_{50}, K_i)	IC_{50}	IC_{50}	IC_{50}
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor

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

Assays Using Rat Uterine Cytosol

Reference	Elsby et al. (2000)	Fang et al. (2001)	Gabbard and Segaloff (1983)
Preparation of receptor			
Species/strain from which receptor obtained	AP rats	Sprague Dawley rats	AXC rats
Age of animals	21-25 days old	245±18 days old	Mature
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	10 days prior to sacrifice	5 days prior to sacrifice
Buffer for preparation of cytosol	TEGM (10 mM Tris, 1.5 mM EDTA, $MgCl_2$ 3 mM, 10% glycerol, pH 7.6)	TEDG (10 mMTris, 1.5 mM EDTA, 10 mM D, 10% glycerol, pH 7.4)	1.5 mM Tris, 1.0 mM EDTA, 20 mM sodium molybdate, pH 7.4
Dilution of tissue with buffer	50 mg/mL buffer	17 mg/mL	10 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	6 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 5 nM - 500 µM	10 µL; 1 nM	n.p.
Specific activity of radioligand	111 Ci/mmol	141 Ci/mmol	53 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	100% ethanol	ethanol
Concentration range of competing ligand	5 nM - 500 µM	n.p.	1 nM to 1 µM
Volume of ER prep used	100 µL	50 µL	100 µL
No. of replicates	n.p.	2	3
No. of times assay repeated	n.p.	2	n.p.
Time of incubation	18 hours	20 hours	2 hours
Temperature of incubation	4°C	4°C	4°C
Measure of nonspecific binding(y/n) and concentration	n.p.	n.p.	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% Hydroxyapatite in TEGM buffer	750 µl cold hydroxyapatite in 50mM Tris pH 7.4	Dextran-coated charcoal (Norite-A + dextran + human gamma globulin)
Incubation time and temperature	n.p.	20 min. at 4°C	15 min. at 4°C
Centrifugation speed	1000xg	600xg	3500xg
Centrifugation time and temperature	10 min. at room temp.	4°C for 5 min.	10 min. at 0°C
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	% Control vs. Concentration (M)	n.p.	% Bound radioactivity vs. Log concentration
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{50}	RBA	relative displacing activity (RDA)
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 Rat Uterine Cytosol

Reference	Jaimez et al. (2000)	Jordan et al. (1986)	Laws et al. (1996)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Sprague Dawley rats	Long Evans rats
Age of animals	Immature	18-21 days	Adult (60 days)
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	11 days prior to sacrifice
Buffer for preparation of cytosol	TEDM (20mM Tris-HCl, 1.5 mM EDTA, 0.25 mM dithiothreitol, 10 µg/mL leupeptine, 10% glycerol, pH 7.4)	TED (10 mMTris, 1.5 mM EDTA, 0.5 mM dithiothourseitol, pH 7.4)	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)
Dilution of tissue with buffer	1:6 (w/v) tissue to buffer ratio	2 uteri/mL buffer	50 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 1 nM	100 µL; $5x10^{-9}$ mol/L	volume n.p.; 1 nM
Specific activity of radioligand	n.p.	51 Ci/mmol	111Ci/nmol
Solvent used to dissolve competing ligand	n.p.	ethanol	20% glycerol/ethanol; TE-G30%-MTG buffer
Concentration range of competing ligand	n.p.	n.p.	0.0001-1000 µM
Volume of ER prep used	n.p.	200 µL	200 µL
No. of replicates	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.
Time of incubation	18 hours	18 hours or 30 min.	30 min.
Temperature of incubation	4ºC	30ºC or 4ºC	30ºC
Measure of nonspecific binding(y/n) and concentration	n.p.	y	y
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Dextran-coated charcoal (250 mg Norite-A + 25 mg Dextran T-70 in 100 mL TEDM buffer)	Dextran-coated charcoal (0.25% Norit A and 0.025% dextran in TED buffer)	250 µL 60% hydroxyapatite in TEG-MTG buffer
Incubation time and temperature	n.p.	20 min. at 4ºC	15 min. at room temp.
Centrifugation speed	800xg	2000xg	1000xg
Centrifugation time and temperature	15 min. at 4ºC	10 min. at 4ºC	10 min. at 4ºC
Data calculations			
Program or method used for calculating data	n.p.	n.p.	Graph Pad Prism
Data plotted as	n.p.	% specific [^{3}H]-E_2 binding in controls vs. log concentration (mol/L)	% Specific binding vs. Competitor (M)
Data format in paper (*e.g.,* IC_{50}, K_i)	RBA	IC_{50}	K_i calculated from EC_{50}
Calculation of RBA	n.p.	IC_{50} E_2/IC_{50} competitor x 100	n.p.

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

Assays Using Rat Uterine Cytosol

Reference	Laws et al. (2000)	Leibl and Spona (1982)	Liu et al. (1994)
Preparation of receptor			
Species/strain from which receptor obtained	Long Evans rats	Sprague Dawley rats	Sprague Dawley rats
Age of animals	Adult (60 days)	Adult (60 - 80 days)	30 days
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	11 days prior to sacrifice	10 days prior to sacrifice	n.p.
Buffer for preparation of cytosol	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)	TMK buffer (10 mM Tris, 1.5 mM MgCl2, 10 mM KCl, pH 7.2)	TEGD (10 mM Tris, 1.5 mM EDTA, 1 mM dithiothourseitol, 10% (v/v) glycerol)
Dilution of tissue with buffer	50 mg/mL buffer	5 uteri/4 mL buffer	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Volume and concentration of radiolabelled 17β *-estradiol*	volume n.p.; 1 nM	volume n.p.; 1 nM	volume n.p.; 1 nM
Specific activity of radioligand	111Ci/nmol	58 Ci/mmol	147 Ci/mmol
Solvent used to dissolve competing ligand	20% glycerol/ethanol; TE-G30%-MTG buffer	ethanol	n.p.
Concentration range of competing ligand	0.0001-1000 µM	1 pM -1 µM	1 nM - 10 µM
Volume of ER prep used	200 µL	n.p.	200 µg protein
No. of replicates	n.p.	2	n.p.
No. of times assay repeated	n.p.	n.p.	3
Time of incubation	30 min.	18 hours	2 hours
Temperature of incubation	30ºC	4ºC	22ºC
Measure of nonspecific binding(y/n) and concentration	y	n.p.	y
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% hydroxyapatite in TEG-MTG buffer	0.25 mL dextran-coated charcoal (0.6% charcoal Norit A, 0.06% dextran T-60)	100 µL dextran-coated charcoal (5% charcoal and 0.5% dextran in TEGD buffer)
Incubation time and temperature	15 min. at room temp.	20 min., 4ºC	20 min., 4ºC
Centrifugation speed	1000xg	3000xg	1500xg
Centrifugation time and temperature	10 min. at 4ºC	10 min.; temp. n.p.	10 min. at 4ºC
Data calculations			
Program or method used for calculating data	Graph Pad Prism	n.p.	n.p.
Data plotted as	% Specific binding vs. Competitor (M)	% Bound vs. Concentration competitor	$[^3H]$-E_2 complex (%) vs. ligand (nM)
Data format in paper (*e.g.,* IC_{50}, K_i)	K_i calculated from EC_{50}	graphical	graphical (EC_{50} estimated)
Calculation of RBA	n.p.	n.p.	n.p.

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

Assays Using Rat Uterine Cytosol

Reference	McBlain (1987)	Nelson et al. (1973)	Olea et al. (1996)
Preparation of receptor			
Species/strain from which receptor obtained	Sprague Dawley rats	Sprague Dawley rats	Rats (otherwise unspecified)
Age of animals	4-5 weeks	2-5 months	Immature
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	(10 mMTris, 1.5 mM EDTA, 12 mM monothioglycerol, 10 mM sodium molybdate, 10% (v/v) glycerol, pH 7.4)	10 mM Tris-HCL+1.5 mM EDTA, pH 7.4	Phosphate buffer
Dilution of tissue with buffer	Uteri powdered under liquid N_2	Uteri from 3-4 mice in 10 mL	n.p.
Protein concentration of cytosol	n.p.	n.p.	2 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β *-estradiol*	volume n.p.; 2 nM	volume n.p.; 2 nM	volume n.p.; 3 nM
Specific activity of radioligand	n.p.	48 Ci/mmole	103 Bq/mmol
Solvent used to dissolve competing ligand	ethanol (final conc. 1.5%)	absolute ethanol	ethanol
Concentration range of competing ligand	0.2 nM-20 µM	0.1 to 500 µM	0.1 nM to 100 µM
Volume of ER prep used	n.p.	400 µg protein	n.p.
No. of replicates	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	3	n.p.
Time of incubation	18 hours	1 hour	16 hours
Temperature of incubation	4°C	4°C	0-4°C
Measure of nonspecific binding(y/n) and concentration	y	n.p.	y
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	0.5 mL dextran-coated charcoal + 1 mg/mL BSA	0.5 mL activated charcoal and 0.5m% Dextran T40 in Tris/HCL	Dextran + charcoal
Incubation time and temperature	10 min.	15 min. at 4°C	n.p.
Centrifugation speed	12,800xg	2000xg for 5 min.	n.p.
Centrifugation time and temperature	5 min.; temp. n.p.	5 min.; temp. n.p.	n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	n.p.
Data plotted as	$[^3H]$-E_2 Bound (% of control) vs. Molar excess of competitor	% Inhibition of $[^3H]$-E_2 binding vs. Concentration (µM)	% Specific binding vs. Concentration (M)
Data format in paper (*e.g.,* IC_{50}, K_i)	IC_{25}, IC_{50}, K_i	graphical	graphical, RBA
Calculation of RBA	n.p.	n.p.	IC_{50} E_2/IC_{50} competitor x 100

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

Assays Using Rat Uterine Cytosol

Reference	Perez et al. (1998)	Qian and Abul-Hajj (1990)	Rijks et al. (1996)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Sprague Dawley rats	Sprague Dawley rats
Age of animals	Immature	Immature	Mature
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	Phosphate buffer	n.p.	ER buffer (10 mMTris.HCl, 1.0 mM EDTA, 1 mM dithiothourseitol, 10 mM sodium molybdate, 0.25M sucrose, pH 7.4)
Dilution of tissue with buffer	n.p.	n.p.	200 mg/mL buffer
Protein concentration of cytosol	2 mg/mL	3.5 mg/mL	3-4 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β -estradiol	volume n.p.; 3 nM	50 µl; concentration n.p.	50 µl; concentration n.p.
Specific activity of radioligand	103 Bq/mmol	91Ci/mmol	4.26 TBq/mmol
Solvent used to dissolve competing ligand	ethanol	n.p.	n.p.
Concentration range of competing ligand	10 pM to 100 µM	1 nM to 3 µM	10 pM to 2 µM
Volume of ER prep used	n.p.	150 µl	50 µL
No. of replicates	n.p.	2	n.p.
No. of times assay repeated	n.p.	n.p.	2
Time of incubation	16 hours	3 hours	18 hours
Temperature of incubation	4°C	4°C	4°C
Measure of nonspecific binding(y/n) and concentration	y	y	n.p.
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	Dextran + charcoal	Dextran-coated charcoal (10 mM Trizma base, 1.0 mM EDTA, 250 mM sucrose, 0.05% dextran, 0.5% charcoal)	n.p.
Incubation time and temperature	n.p.	15 min. 4°C	n.p.
Centrifugation speed	n.p.	2000xg	n.p.
Centrifugation time and temperature	n.p.	0°C for 5 min.	n.p.
Data calculations			
Program or method used for calculating data	n.p.	n.p.	LIGAND computer program
Data plotted as	% Specific binding vs. Concentration (M)	n.p.	% Bound vs. Concentration competitor (nM)
Data format in paper (*e.g.,* IC_{50}, K_i)	graphical, RBA	RBA	K_i
Calculation of RBA	IC_{50} E_2/IC_{50} competitor x 100	IC_{50} E_2/IC_{50} competitor x 100	K_i reference steroid/ K_i competitor x 100

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

Assays Using Rat Uterine Cytosol

Reference	Routledge et al. (1998)	Waller et al. (1996)	Zacharewski et al. (1998)
Preparation of receptor			
Species/strain from which receptor obtained	Rats (otherwise unspecified)	Long Evans rats	Sprague Dawley rats
Age of animals	8-10 weeks	Adult (60 days)	22 day old
Source of receptor	Uterus	Uterus	Uterus
Isolated preparation	Cytosol	Cytosol	Cytosol
When ovariectomized	n.p.	11 days prior to sacrifice	n.p.
Buffer for preparation of cytosol	TEGM (10 mMTris, 1.5 mM EDTA, 3 mM $MgCl_2$, 10% glycerol, pH 7.4)	TE-G30%-MTG (50 mM Tris, 0.9 mM EDTA, 30% (v/v) glycerol, 0.15% (v/v) monothioglycerol, pH 7.4)	TEGD buffer (10 mMTris base 1.5 mM EDTA, 1 mM dithiothourseitol, 10% glycerol, pH 7.6)
Dilution of tissue with buffer	50 mg/mL	50 mg/mL buffer	200 mg/mL buffer
Protein concentration of cytosol	n.p.	n.p.	2 mg/mL
Competitive binding assay			
Volume and concentration of radiolabelled 17β *-estradiol*	volume n.p.; 5 nM	volume n.p.; 1 nM	30 µL; 1 nM
Specific activity of radioligand	110 Ci/mmol	111Ci/nmol	84 Ci/mmol
Solvent used to dissolve competing ligand	n.p.	20% glycerol/ethanol; TE-G30%-MTG buffer	dimethyl sulfoxide
Concentration range of competing ligand	0.5 nM -500 µM	0.0001-1000 µM	1-1000 µM
Volume of ER prep used	100 µL	200 µL	240 µL
No. of replicates	2	n.p.	2
No. of times assay repeated	n.p.	n.p.	3
Time of incubation	18 hours	30 min.	30 min.
Temperature of incubation	4ºC	30ºC	30ºC
Measure of nonspecific binding(y/n) and concentration	n.p.	y	y, 30 µL
Separation of ligand			
Type of slurry (hydroxyapatite, charcoal, protamine sulfate)	250 µL 60% hydroxyapatite	250 µL 60% hydroxyapatite in TEG-MTG buffer	125 µL 60% hydroxyapatite in TEGD buffer
Incubation time and temperature	n.p.	15 min. at room temp.	n.p.
Centrifugation speed	1,000xg	1000xg	n.p.
Centrifugation time and temperature	10 min.	10 min. at room temp.	n.p.
Data calculations			
Program or method used for calculating data	Ligand Competition Analysis Software (Lundon Software, Chagrin Falls, OH)	one side competitive binding curves (Graph Pad Prism)	n.p.
Data plotted as	% Control vs. Molarity	% Specific binding vs. Competitor (M)	$[^3H]$-E_2 Bound vs. Log concentration of unlabeled competitor (M)
Data format in paper (*e.g.,* IC_{50}, K_i)	graphical	K_i calculated from EC_{50}	IC_{50}
Calculation of RBA	n.p.	n.p.	n.p.

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