Assays Using Rat Prostate or Epididymal Cytosol

Reference	Danzo (1997)	Kelce et al. (1994)	Kelce et al. (1995)
Preparation of receptor			
Source of receptor	Rat (otherwise unspecified)	Sprague Dawley rat	Rat (otherwise unspecified)
Tissue	prostate	epididymis	ventral prostate
Age of animals	n.p.	120 -150 days	n.p.
When castrated	n.p.	24 hours before sacrifice	24 hours before sacrifice
Diet of animals	n.p.	Purina Lab Chow - 5001	n.p.
Environment	n.p.	22° C, 40-50% humidity	n.p.
Lighting	n.p.	14 hours light:10 hours dark	n.p.
Buffer for preparation of cytosol	n.p.	TEDG, pH 7.4	n.p.
Dilution of tissue with buffer	n.p.	5 ml/gm	n.p.
Homogenization	n.p.	Polytron	n.p.
Centrifugation	n.p.	30000xg, 4° C, 10 min	n.p.
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Reference ligand	5α-Dihydrotestosterone	R1881	R1881
Volume and concentration of reference ligand	n.p.	volume n.p.; 0.5 to 20.0 nM	volume n.p.; 3 nM
Specific activity of labelled reference ligand	n.p.	86 Ci/mmol	n.p.
Volume and concentration of cold ligand	n.p.	volume n.p.; .01 - 1000 nM or .01 - 400 nM	volume n.p.; 100 nM
Final concentration of reference ligand	n.p.	n.p.	103 nM
Concentration range of competing ligand	n.p.	n.p.	0-100; 0-50; 0-1.2 µM
Volume of cytosol	n.p.	300 µl	n.p.
Volume of buffer	n.p.	n.p.	n.p.
Type of buffer used	n.p.	n.p.	n.p.
Replicates	2	n.p.	n.p.
Time of incubation	n.p.	20 hours	20 hours
Temperature of incubation	n.p.	4° C	4° C

Assays Using Rat Prostate or Epididymal Cytosol

Reference	Danzo (1997)	Kelce et al. (1994)	Kelce et al. (1995)
Separation of ligand			
Type of slurry	dextran-charcoal	hydroxylapatite	n.p.
Buffer for slurry	n.p.	Tris, pH 7.4	n.p.
Incubation time and temperature	n.p.	20 min, temp. n.p.	n.p.
Centrifugation speed	n.p.	600xg	n.p.
Centrifugation time and temperature	n.p.	2 min; 4º C	n.p.
Resuspension volume and buffer for pellet	n.p.	Tris, pH 7.4	n.p.
No. of washes	n.p.	3	n.p.
Extraction of label	n.p.	2 ml ethanol	n.p.
Incubation time and temperature	n.p.	10 min	n.p.
Vortexing during incubation time	n.p.	yes	n.p.
Centrifugation time and temperature	n.p.	n.p.	n.p.
Measurement of Binding			
Volume added for reading	n.p.	n.p.	n.p.
Volume of fluor	n.p.	15 ml scintillation fluid	n.p.
Type of fluor	n.p.	n.p.	n.p.
Instrumentation	n.p.	n.p.	n.p.
Measurement	n.p.	n.p.	n.p.
Blank without competitor	n.p.	n.p.	n.p.
Reading of blank	n.p.	n.p.	n.p.
Blank subtracted?	n.p.	n.p.	n.p.
Range of standard curve of reference ligand	n.p.	.01-1000 nM; .01-400 nM	n.p.
Nonspecific binding measured?	n.p.	yes	n.p.
Subtraction of nonspecific binding	n.p.	yes	n.p.
Data calculations			
Data plotted as	% Inhibition	Scatchard plots	Scatchard plots
Data calculated	n.p.	n.p.	n.p.
Calculation of RBA	from bar graph	n.p.	n.p.
Test substances			
Solvent used	n.p.	n.p.	n.p.
No. of samples/ dose	n.p.	2	n.p.
No. of times assay repeated	varies from 3 to 8 depending on substance	n.p.	n.p.

Abbreviations: n.a. = not applicable; No. = number; n.p. = not provided; RBA = relative binding affinity

Assays Using Rat Prostate or Epididymal Cytosol

Reference	Lambright et al. (2000)	Schilling and Liao (1984)	Teutsch et al. (1994)
Preparation of receptor			
Source of receptor	Sprague Dawley rat	Sprague Dawley rat	Sprague Dawley rat
Tissue	ventral prostate	ventral prostate	prostate
Age of animals	90 days	n.p.	n.p.
When castrated	24 hours before sacrifice	18 hours before sacrifice	24 hours before sacrifice
Diet of animals	n.p.	n.p.	n.p.
Environment	n.p.	n.p.	n.p.
Lighting	n.p.	n.p.	n.p.
Buffer for preparation of cytosol	TEDG	Dulbecco's MEM, Hepes, pH 7.5	Tris, DTT, phenylmethylsulfonyl fluoride, molybdate, pH 7.4
Dilution of tissue with buffer	10 ml/gm	n.p.	n.p.
Homogenization	Polytron	Potter-Elvejhem	n.p.
Centrifugation	30000xg	220,000xg, 45 min	209,000xg, 30 min, 0-4° C
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Reference ligand	R1881	Mibolerone	Testosterone
Volume and concentration of reference ligand	volume n.p.; 5 nM	volume n.p.; 10 - 30 nM	volume n.p.; 2.5 or 5 mM
Specific activity of labelled reference ligand	n.p.	80.9 Ci/mmol	54 Ci/mM
Volume and concentration of cold ligand	n.p.	n.p.	n.p.
Final concentration of reference ligand	n.p.	10 nM	0.1 - 20 nM
Concentration range of competing ligand	n.p.	50, 100, 300 nM	n.p.
Volume of cytosol	n.p.	n.p.	0.1 ml
Volume of buffer	n.p.	n.p.	n.p.
Type of buffer used	n.p.	n.p.	n.p.
Replicates	n.p.	n.p.	n.p.
Time of incubation	2 hours	n.p.	24 hours
Temperature of incubation	37° C	n.p.	0° C

Assays Using Rat Prostate or Epididymal Cytosol

Reference	Lambright et al. (2000)	Schilling and Liao (1984)	Teutsch et al. (1994)
Separation of ligand			
Type of slurry	n.p.	hydroxyapatite	dextran-charcoal
Buffer for slurry	n.p.	Tris, PO_4, pH 7.2	Tris, DTT, phenylmethylsulfonyl fluoride, molybdate, pH 7.4
Incubation time and temperature	n.p.	10 min, 0º C	10 min, 0-4º C
Centrifugation speed	n.p.	filtered	800xg
Centrifugation time and temperature	n.p.	n.a.	10 min, 0-4º C
Resuspension volume and buffer for pellet	n.p.	scintillation fluid	n.p.
No. of washes	n.p.	5	n.p.
Extraction of label	n.p.	scintillation fluid	n.p.
Incubation time and temperature	n.p.	n.p.	n.p.
Vortexing during incubation time	n.p.	n.p.	n.p.
Centrifugation time and temperature	n.p.	n.p.	n.p.
Measurement of Binding			
Volume added for reading	n.p.	n.p.	0.1 ml
Volume of fluor	n.p.	n.p.	n.p.
Type of fluor	n.p.	toluene, Triton-X100	n.p.
Instrumentation	n.p.	n.p.	n.p.
Measurement	n.p.	n.p.	n.p.
Blank without competitor	n.p.	n.p.	n.p.
Reading of blank	n.p.	n.p.	n.p.
Blank subtracted?	n.p.	no	n.p.
Range of standard curve of reference ligand	n.p.	10 nM	n.p.
Nonspecific binding measured?	n.p.	n.p.	n.p.
Subtraction of nonspecific binding	n.p.	n.p.	n.p.
Data calculations			
Data plotted as	n.p.	n.p.	Scatchard analysis
Data calculated	n.p.	% binding	n.p.
Calculation of RBA	n.p.	calculated from % binding	yes
Test substances			
Solvent used	n.p.	ethanol	n.p.
No. of samples/ dose	n.p.	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.	n.p.

Abbreviations: n.a. = not applicable; No. = number; n.p. = not provided; RBA = relative binding affinity

Assays Using Rat Prostate or Epididymal Cytosol

Reference	Van Dort et al. (2000)	Waller et al. (1996)	Wilson and French (1976)
Preparation of receptor			
Source of receptor	Wistar rat	Sprague Dawley rat	Sprague Dawley or Osborne-Mendel rat
Tissue	ventral prostate	epididymis	prostate
Age of animals	n.p.	120 -150 days	n.p.
When castrated	n.p.	24 hours before sacrifice	24 hours before sacrifice
Diet of animals	n.p.	Purina Lab chow - 5001	n.p.
Environment	n.p.	22° C, 40-50% humidity	n.p.
Lighting	n.p.	14 hours light:10 hours dark	n.p.
Buffer for preparation of cytosol	PO_4 with protease inhibitor and triamcinolone acetate, pH 7.2	TEDG, pH 7.4	Tris-EDTA-glycerol, pH 7.5
Dilution of tissue with buffer	n.p.	5 ml/gm	n.p.
Homogenization	n.p.	Polytron	Ultra turrax, set at 7
Centrifugation	n.p.	30000xg, 4° C, 10 min	105000xg, 75 min
Protein concentration of cytosol	n.p.	n.p.	n.p.
Competitive binding assay			
Reference ligand	Mibolerone	R1881	5α-Dihydrotestosterone
Volume and concentration of reference ligand	volume n.p.; 2 nM	n.p.	volume n.p.; 15-20 nM
Specific activity of labelled reference ligand	n.p.	n.p.	80 Ci/mmol
Volume and concentration of cold ligand	volume n.p.; 0.3 - 100 nM	n.p.	volume n.p.; 2000 nM
Final concentration of reference ligand	2 nM	n.p.	2020 nM
Concentration range of competing ligand	0.3 - 100 nM	n.p.	20 - 2000 nM
Volume of cytosol	n.p.	300 µl	n.p.
Volume of buffer	n.p.	n.p.	n.p.
Type of buffer used	PO_4 with protease inhibitor and triamcinolone acetate, pH 7.2	n.p.	n.p.
Replicates	duplicate	n.p.	n.p.
Time of incubation	18 hours	20 hours	18 - 20 hours
Temperature of incubation	4° C	4° C	0° C

Assays Using Rat Prostate or Epididymal Cytosol

Reference	Van Dort et al. (2000)	Waller et al. (1996)	Wilson and French (1976)
Separation of ligand			
Type of slurry	hydroxyapatite	hydroxylapatite	charcoal-dextran
Buffer for slurry	n.p.	Tris, pH 7.4	Tris-EDTA, pH 7.5
Incubation time and temperature	15 min, temp. n.p.	20 min, temp. n.p.	20 min, 0º C
Centrifugation speed	n.p.	600xg	2000xg
Centrifugation time and temperature	n.p.	2 min; 4 C	15 min
Resuspension volume and buffer for pellet	n.p.	Tris, pH 7.4	n.p.
No. of washes	n.p.	3	n.p.
Extraction of label	centrifugation	2 ml ethanol	n.p.
Incubation time and temperature	n.p.	10 min	n.p.
Vortexing during incubation time	n.p.	yes	n.p.
Centrifugation time and temperature	n.p.	n.p.	n.p.
Measurement of Binding			
Volume added for reading	n.p.	n.p.	0.5 ml
Volume of fluor	n.p.	15 ml scintillation fluid	5 ml
Type of fluor	n.p.	n.p.	Aquasol/toluene, 1:1
Instrumentation	n.p.	n.p.	n.p.
Measurement	n.p.	n.p.	n.p.
Blank without competitor	n.p.	n.p.	n.p.
Reading of blank	n.p.	n.p.	n.p.
Blank subtracted?	n.p.	n.p.	n.p.
Range of standard curve of reference ligand	n.p.	.01-1000 nM; .01-400 nM	n.p.
Nonspecific binding measured?	n.p.	yes	n.p.
Subtraction of nonspecific binding	n.p.	yes	n.p.
Data calculations			
Data plotted as	n.p.	Scatchard plots	n.p.
Data calculated	Ki	n.p.	from binding graph
Calculation of RBA	n.p.	n.p.	from binding graph
Test substances			
Solvent used	n.p.	n.p.	n.p.
No. of samples/ dose	2	2	n.p.
No. of times assay repeated	3	n.p.	n.p.

Abbreviations: n.a. = not applicable; No. = number; n.p. = not provided; RBA = relative binding affinity