Miscellaneous AR Binding Assays

Reference	Bauer et al. (1998)	Bauer et al. (2000)
Preparation of Receptor		
Animal or cell line	Prepubertal calves	Sf9 insect cells transfected with recombinant baculovirus
Source of receptor	Uterus	Human recombinant AR
Age of animals	n.p.	n.a.
When castrated	n.a.	n.a.
Diet of animals	n.p.	n.a.
Environment	n.p.	n.a.
Lighting	n.p.	n.a.
Buffer for preparation of cytosol	Tris-EDTA-glycerol-protease inhibitor, pH 7.4	n.a.
Dilution of tissue with buffer	1 to 4	n.a.
Homogenization	Ultraturrax	n.a.
Centifugation	285,000xg, 1 hr, 4° C	n.a.
Storage	-60° C	n.p.
Protein concentration of cytosol	n.p.	n.a.
Preparation of Cells for Assay		
Whole cells/ cell homogenate	n.a.	semi-purified recombinant protein
Serum source	n.a.	n.a.
Serum stripping method	n.a.	n.a.
Residual androgen in serum	n.a.	n.a.
No. treated cells/No. or weight of cells homogenized	n.a.	n.a.
Treatment vessel used	n.a.	n.a.
Preparation of cell homogenate	n.a.	n.a.
volume	n.a.	n.a.
buffer	n.a.	n.a.
method	n.a.	n.a.
time; temperature	n.a.	n.a.
Centrifugation of homogenate (time, speed, temperature)	n.a.	n.a.
Protein concentration of cytosol	n.a.	n.a.
Storage	n.a.	n.a.
Final protein concentration	n.a.	n.a.
Test chemical solvent	n.a.	n.a.
Separation of bound hormone	n.a.	n.a.
Competitive binding assay		
Reference ligand	5α-Dihydrotestosterone	5α-Dihydrotestosterone
Volume and concentration of reference ligand	4 nM	0.4 nM
Specific activity of labelled reference ligand	n.p.	4.70 TBq/mmol
ligand	n.p.	n.p.
ligand	4 nM	0.4 nM
Volume of competing ligand	n.p.	

Miscellaneous AR Binding Assays

Reference	Bauer et al. (1998)	Bauer et al. (2000)
Concentration range of competing ligand	n.p.	n.p.
Volume of cytosol	0.5 ml	0.5 ml
Volume of buffer	n.p.	n.p.
Type of buffer used	n.p.	phosphate, pH 7.2 + protease inhibitor
Replicates	n.p.	triplicate
Time of incubation	16 hr	16 hr
Temperature of incubation	0-4 C	0-4 C
Separation of ligand		
Volume and type of slurry	100 ul dextran-charcoal	dextran-charcoal
Buffer for slurry	Tris-EDTA-glycerol-protease inhibitor, pH 7.4	phosphate, pH 7.2 + protease inhibitor
Incubation time and temp	5 min, 4° C	5 min, 4° C
Time of vortexing	n.p.	n.p.
Centrifugation speed	2000xg	2000xg
Centrifugation time and temperature	15 min, 4° C	15 min, 4° C
Resuspension volume and buffer for pellet	n.p.	3 ml
No. of washes	1	n.p.
Extraction of label	n.a.	
Incubation time and temperature	n.a.	n.p.
Vortexing during incubation time	n.a.	n.p.
Centrifugation time and temperature	n.a.	n.p.
Volume added for reading	0.4 ml	n.p.
Volume of fluor	3 ml	3 ml
Type of fluor	Xylofluor	Xyloflour
Instrumentation	n.p.	n.p.
Measurement	n.p.	n.p.
Blank without competitor	n.p.	n.p.
Reading of blank	n.p.	n.p.
Blank subtracted?	n.p.	n.p.
Range of standard curve of reference ligand	n.a.	n.p.
Nonspecific binding measured?		n.p.
Subtraction of nonspecific binding	n.p.	n.p.
Data calculations		
Data plotted as	nonlinear, log progression, 4 parameters	Scatchard Plots; Sigma plot
Data calculated	Ki	Ki
Calculation of RBA	from Scatchard plot	yes
Test chemicals		
Solvent used	n.p.	n.p.
No. of samples/ dose	n.p.	3
No. of times assay repeated	n.p.	n.p.
Abbreviations: n.a. = not applicable; n.p. = not provided; RBA = relative binding affinity		

Miscellaneous AR Binding Assays

Reference	Sonnenschein et al. (1989)	Takeo and Yamashita (2000)
Preparation of Receptor		
Animal or cell line	LnCaP-FGC cells	Transfected COS-1 cells
Source of receptor	Human mutant AR from metastatic lymph node of a primary prostate adenocarcinoma	Rainbow trout ARα expression vector
Age of animals	n.a.	n.a.
When castrated	n.a.	n.a.
Diet of animals	n.a.	n.a.
Environment	n.a.	n.a.
Lighting	n.a.	n.a.
Buffer for preparation of cytosol	n.a.	n.a.
Dilution of tissue with buffer	n.a.	n.a.
Homogenization	n.a.	n.a.
Centifugation	n.a.	n.a.
Storage	n.a.	n.p.
Protein concentration of cytosol	n.a.	n.a.
Preparation of Cells for Assay		
Whole cells/ cell homogenate	cytosol	cytosol
Serum source	fetal bovine serum (5%)	n.p.
Serum stripping method	n.p.	n.p.
Residual androgen in serum	n.p.	n.p.
No. treated cells/No. or weight of cells homogenized	n.p.	n.p.
Treatment vessel used	n.p.	n.p.
Preparation of cell homogenate		n.p.
volume	n.p.	n.p.
buffer	Tris-EDTA-KCl, pH 7.4	n.p.
method	sonication	n.p.
time; temperature	n.p.	n.p.
Centrifugation of homogenate (time, speed, temperature)	105,000 x g, 45 min	n.p.
Protein concentration of cytosol	n.p.	n.p.
Storage	n.p.	n.p.
Final protein concentration	n.p.	n.p.
Test chemical solvent	n.p.	n.p.
Separation of bound hormone	n.p.	n.p.
Competitive binding assay		
Reference ligand	Testosterone	Mibolerone
Volume and concentration of reference ligand	6 nM	1 nM
Specific activity of labelled reference ligand	3.1 TBq/mmol	n.p.
ligand	n.p.	n.p.
ligand	6 nM	n.p.
Volume of competing ligand	n.p.	n.p.

Miscellaneous AR Binding Assays

Reference	Sonnenschein et al. (1989)	Takeo and Yamashita (2000)
Concentration range of competing ligand	0.5 - 5000 nM	1-1000 nM
Volume of cytosol	n.p.	n.a.
Volume of buffer	n.p.	n.p.
Type of buffer used	n.p.	n.p.
Replicates	n.p.	n.p.
Time of incubation	n.p.	5 hr
Temperature of incubation	n.p.	4 C
Separation of ligand		
Volume and type of slurry	n.p.	dextran-charcoal, 50 µl
Buffer for slurry	n.p.	Tris, pH 7.2
Incubation time and temp	n.p.	5 min, 0° C
Time of vortexing	n.p.	n.p.
Centrifugation speed	n.p.	2000xg
Centrifugation time and temperature	n.p.	10 min, 0° C
Resuspension volume and buffer for pellet	n.p.	5 ml
No. of washes	n.p.	1
Extraction of label	n.p.	n.p.
Incubation time and temperature	n.p.	n.p.
Vortexing during incubation time	n.p.	n.p.
Centrifugation time and temperature	n.p.	n.p.
Volume added for reading	n.p.	n.p.
Volume of fluor	n.p.	5 ml
Type of fluor	n.p.	n.p.
Instrumentation	n.p.	n.p.
Measurement	n.p.	n.p.
Blank without competitor	n.p.	n.p.
Reading of blank	n.p.	n.p.
Blank subtracted?	n.p.	n.p.
Range of standard curve of reference ligand	n.p.	n.p.
Nonspecific binding measured?	n.p.	n.p.
Subtraction of nonspecific binding	n.p.	n.p.
Data calculations		
Data plotted as	Cell number(10^5)/well vs. Steroid concentration (M)	Graphpad prism software
Data calculated	I_{50}	n.p.
Calculation of RBA	from I_{50} (data not presented)	Estimated from competitor binding graph
Test chemicals		
Solvent used	n.p.	n.p.
No. of samples/ dose	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.

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