Assays Using Human Genital Fibroblast Cells

Reference	Breiner et al. (1986)	Brown et al. (1981)
Preparation of receptor		
Source of receptor	Human primary genital skin fibroblasts	Human penile fibroblast explants
Whole cells/ cell homogenate	whole cells	whole cells
Serum source	Fetal calf (10%)	Fetal bovine
Serum stripping method	none	n.p.
Residual androgen in serum	n.p.	n.p.
No. of treated cells/No. or weight of cells homogenized	monolayer	confluent
Treatment vessel used	60 x 15 mm falcon	culture plates
Competitive binding assay		
Reference ligand	5α-Dihydrotestosterone	5α-Dihydrotestosterone
Volume and concentration of reference ligand	2 nM	2 nM
Specific activity of labelled reference ligand	123 - 153 Ci/mmol	131 Ci/mmol
Volume and concentration of cold ligand	n.p.	2-1000 nM
Final concentration of reference ligand	2 nM	n.p.
Volume of competing ligand	n.p.	n.p.
Concentration range of competing ligand	n.p.	1 - 1000 nM
Volume of cytosol	n.a.	n.a.
Volume of buffer	3 ml	n.p.
Type of buffer used	Eagle's minimal essential medium	serum-free MEM
Replicates	duplicate	single
Time of incubation	60 min	45 min
Temperature of incubation	37° C	37° C
Separation of ligand		
Volume and type of slurry	dextran-charcoal	dextran-charcoal
Buffer for slurry	Tris-EDTA-KCl, pH 7.4	Tris-EDTA, pH 7.4
Incubation time and temperature	10 min; temp n.p.	10 min, 0-4° C
Time of vortexing	10 min	10 min
Centrifugation speed	2500 x g	2000xg
Centrifugation time and temperature	15 min; time n.p.	5 min, 0-4° C
Resuspension volume and buffer for pellet	n.p.	n.p.
No. of washes	n.p.	n.p.
Extraction of label	supernatant counted	n.p.
Incubation time and temperature	n.a.	n.p.
Volume of fluor	n.p.	n.p.
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.	yes
Subtraction of nonspecific binding	n.p.	yes

Assays Using Human Genital Fibroblast Cells

Reference	Breiner et al. (1986)	Brown et al. (1981)
Data calculations		
Data plotted as	%[^{3}H]-DHT bound vs. Competitor concentration (M)	n.p.
Data calculated	Ki	IC_{50} (data not presented)
Calculation of RBA	from IC_{50} (data not presented)	yes
Test substances		
Solvent used	n.p.	n.p.
No. of samples/dose	2	1
No. of times assay repeated	n.p.	n.p.
Abbreviations: n.a. = not applicable; No. = number; n.p. = not provided; RBA = relative binding affinity		

Assays Using Human Genital Fibroblast Cells

Reference	Eil and Edelson (1984)
Preparation of receptor	
Source of receptor	Human newborn foreskin fibroblasts
Whole cells/ cell homogenate	whole cells
Serum source	Fetal calf
Serum stripping method	n.p.
Residual androgen in serum	n.p.
No. of treated cells/No. or weight of cells homogenized	0.5 - 2.0x10^{-6} cells/tube
Treatment vessel used	tissue culture flasks
Competitive binding assay	
Reference ligand	R1881; occasionally 5α-Dihydrotestosterone
Volume and concentration of reference ligand	0.5 µM R1881; 1.0 - 1.2 nM DHT
Specific activity of labelled reference ligand	n.p.
Volume and concentration of cold ligand	n.p.
Final concentration of reference ligand	n.p.
Volume of competing ligand	n.p.
Concentration range of competing ligand	n.p.
Volume of cytosol	n.a.
Volume of buffer	n.p.
Type of buffer used	EMEM medium
Replicates	n.p.
Time of incubation	60 min
Temperature of incubation	22º C
Separation of ligand	
Volume and type of slurry	n.p.
Buffer for slurry	n.p.
Incubation time and temperature	n.p.
Time of vortexing	n.p.
Centrifugation speed	n.p.
Centrifugation time and temperature	n.p.
Resuspension volume and buffer for pellet	n.p.
No. of washes	n.p.
Extraction of label	n.p.
Incubation time and temperature	n.p.
Volume of fluor	n.p.
Type of fluor	n.p.
Instrumentation	n.p.
Measurement	n.p.
Blank without competitor	n.p.
Reading of blank	n.p.
Blank subtracted?	n.p.
Range of standard curve of reference ligand	1.0 - 1.2 nM
Nonspecific binding measured?	n.p.
Subtraction of nonspecific binding	n.p.

Assays Using Human Genital Fibroblast Cells

Reference	Eil and Edelson (1984)
Data calculations	
Data plotted as	Scatchard plots
Data calculated	Ki
Calculation of RBA	from Ki
Test substances	
Solvent used	ethanol
No. of samples/dose	n.p.
No. of times assay repeated	n.p.

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