Assays Using MCF-7 Cells

Reference	Deckers et al. (2000)	Schoonen et al. (1995)
Characteristics of Cells		
Cell line	MCF-7	MCF-7
Source of cell line	human mammary tumor	human mammary tumor
Whole cells/cytosol	cytosol	cytosol
Preparation of Cells for Assay		
Serum source	fetal calf serum	fetal calf serum
Serum stripping method	charcoal treated serum	charcoal treated serum
Residual androgen in serum	n.p.	n.p.
No. treated cells/No. or weight of cells homogenized	1 gm cells	1 gm cells
Treatment vessel used	n.p.	n.p.
Preparation of cell homogenate		
volume	5 ml	5 ml
buffer	TrisHCl pH 7.4 + EDTA, dithioerythritol, molybdate	TrisHCl pH 7.4 + EDTA, dithioerythritol, molybdate
method	Dounce homogenizer	Dounce homogenizer
time; temperature	n.p.	n.p.
Centrifugation of homogenate	1,000,000N/kg	1,000,000N/kg
Protein concentration of cytosol	n.p.	n.p.
Storage	n.p.	n.p.
Final protein concentration	n.p.	n.p.
Competitive binding assay		
Reference ligand	5α-Dihydrotestosterone	5α-Dihydrotestosterone
Volume and concentration of reference ligand	1.9 nM	1.9 nM
Specific activity of labelled reference ligand	5.3 TBq/mmol	4070 GBq/mmol
Volume and concentration of cold ligand	n.p.	n.p.
Final concentration of reference ligand	1.9 nM	1.9 nM
Volume of competing ligand	n.p.	n.p.
Concentration range of competing ligand	0.1 - 10000 nM	0.1 - 10000 nM
Volume of cytosol	1:5 dilution	1:5 dilution
Volume of buffer	n.p.	n.p.
Type of buffer used	n.p.	n.p.
Replicates	6 or more	2
Time of incubation	overnight	overnight
Temperature of incubation	4° C	4° C

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Reference	Deckers et al. (2000)	Schoonen et al. (1995)
Separation of ligand		
Volume and type of slurry	dextran-charcoal	dextran-charcoal
Buffer for slurry	TrisHCl pH 7.4 + EDTA, dithioerythritol, molybdate	TrisHCl pH 7.4 + EDTA, dithioerythritol, molybdate
Incubation time and temperature	10 min, 4° C	10 min, 4° C
Time of vortexing	n.a.	n.a.
Centrifugation speed	8000N/kg	8000N/kg
Centrifugation time and temperature	5 min	5 min
Resuspension volume and buffer for pellet	n.p.	n.p.
No. of washes	n.p.	n.p.
Extraction of label	centrifugation	centrifugation
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.	n.p.
Type of fluor	n.p.	n.p.
Instrumentation	Topcount microplate scintillation counter	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?	yes	n.p.
Subtraction of nonspecific binding	yes	n.p.
Data calculations		
Data plotted as	n.p.	n.p.
Data calculated	specific binding	IC_{50}
Calculation of RBA	yes	yes
Test substances		
Solvent used	ethanol	ethanol
No. of samples/ dose	n.p.	n.p.
No. of times assay repeated	from 6 to 34	2
Abbreviations: n.a. = not applicable; n.p. = not provided; RBA = relative binding affinity		