AR TA Assays Using CHO Cells

Reference	Bonefeld-Jorgensen et al. (2001)	Deckers et al. (2000)
Characteristics of Cell Line		
Name of cell line	CHO-K1	CHO-K1 clone 1G12-A5-CA
Cell source	Chinese hamster ovary	Chinese hamster ovary
Transfection of Cells with Plasmids		
Stable or transient tranfection	Transient AR/Transient reporter	Stable AR/Stable reporter
AR expression vector	pSVAR0	hAR-MMTV-LUC
AR source	human	human
Reporter vector	pMMTV-LUC	hAR-MMTV-LUC
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	none
Endpoint measured for cell toxicity	n.a.	n.a.
Preparation of Cells for Assay		
Transient	Cells treated with test compound just prior to transfection	
Pregrowth of cells before transient transfection	24 hours pregrowth before treatment with test substance and transfection	n.p.
Time from transient transfection to treatment of cells	n.a.	n.p.
Stable		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Test substance solvent	Ethanol	Ethanol
No. of replicates	4	n.p.
No. of times assay repeated	3	from 1 to 30
Test substance incubation time	24 hours	16 hours
Agonism assay		
Reference ligand	Methyltrienolone	5α-Dihydrotestosterone
Final concentration of reference ligand	100 nM	n.p.
Antagonism assay		
Reference ligand	Methyltrienolone	n.a.
Final concentration of reference ligand	0.1 nM	n.a.

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using CHO Cells

Reference	Deslypere et al. (1992)	Otsuka Pharmaceutical Co. (2001)
Characteristics of Cell Line		
Name of cell line	CHO	CHO-K1 (AR-EcoScreen™)
Cell source	Chinese hamster ovary	Chinese hamster ovary
Transfection of Cells with Plasmids		
Stable or transient tranfection	Transient AR/Transient reporter	Stable AR/Stable reporter
AR expression vector	pCMV3.1.hAR	pZeoSV2AR
AR source	human	n.p.
Reporter vector	MMTV-CAT	pIND ARE B10
Endpoint measured	CAT activity	Luciferase activity
Plasmid transfected for cell toxicity	SV40-pCH110 (β-gal)	none
Endpoint measured for cell toxicity	β-galactosidase activity	n.a.
Preparation of Cells for Assay		
Transient		
Pregrowth of cells before transient transfection	Within 24 hours	n.a.
Time from transient transfection to treatment of cells	24 hours	n.a.
Stable		
Plating time prior to treatment with test substance	n.a.	24 hours
Transcriptional activation assay		
Test substance solvent	n.p.	DMEM/F12 medium
No. of replicates	2	n.p.
No. of times assay repeated	1 to 12	n.p.
Test substance incubation time	12, 24, 36, 48 hours	24 hours
Agonism assay		
Reference ligand	Testosterone	5α-Dihydrotestosterone
Final concentration of reference ligand	n.p.	.001 μM
Antagonism assay		
Reference ligand	n.a.	5α-Dihydrotestosterone
Final concentration of reference ligand	n.a.	n.p.

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using CHO Cells

Reference	Otsuka Pharmaceutical Co. (2001)	Vinggaard et al. (1999)
Characteristics of Cell Line		
Name of cell line	CHO-K1 (EcoScreen™ High throughput transfection assay)	CHO-K1
Cell source	Chinese hamster ovary	Chinese hamster ovary
Transfection of Cells with Plasmids		
Stable or transient tranfection	Transient AR/Transient reporter	Transient AR/Transient reporter
AR expression vector	pZeoSV2AR	pSVAR0
AR source	n.p.	human
Reporter vector	pIND ARE B10	MMTV-LUC
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	pcDNA-EGFP	none
Endpoint measured for cell toxicity	Fluorescence	n.a.
Preparation of Cells for Assay		
Transient		Cells treated with test compound just prior to transfection
Pregrowth of cells before transient transfection	24 hours	24 hours pregrowth before treatment with test substance and transfection
Time from transient transfection to treatment of cells	3 hours	n.p.
Stable		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional activation assay		
Test substance solvent	DMSO + culture medium	DMEM/F12 medium
No. of replicates	4	4
No. of times assay repeated	2	3 to 5
Test substance incubation time	16 to 24 hours	24 hours
Agonism assay		
Reference ligand	n.a.	Methyltrienolone
Final concentration of reference ligand	n.a.	n.p.
Antagonism assay		
Reference ligand	5α-Dihydrotestosterone	Methyltrienolone
Final concentration of reference ligand	5 nM	0.1 nM

Abbreviations: n.a. = not applicable;
n.p. = not provided

AR TA Assays Using CHO Cells

Reference	Vinggaard et al. (2000)
Characteristics of Cell Line	
Name of cell line	CHO-K1
Cell source	Chinese hamster ovary
Transfection of Cells with Plasmids	
Stable or transient tranfection	Transient AR/Transient reporter
AR expression vector	pSVAR0
AR source	human
Reporter vector	MMTV-LUC
Endpoint measured	Luciferase activity
Plasmid transfected for cell toxicity	none
Endpoint measured for cell toxicity	n.a.
Preparation of Cells for Assay	
Transient	Cells treated with test compound just prior to transfection
Pregrowth of cells before transient transfection	24 hours pregrowth before treatment with test substance and transfection
Time from transient transfection to treatment of cells	n.p.
Stable	
Plating time prior to treatment with test substance	n.a.
Transcriptional activation assay	
Test substance solvent	DMEM/F12 medium
No. of replicates	4
No. of times assay repeated	3
Test substance incubation time	24 hours
Agonism assay	
Reference ligand	Methyltrienolone
Final concentration of reference ligand	n.p.
Antagonism assay	
Reference ligand	Methyltrienolone
Final concentration of reference ligand	0.1 nM

Abbreviations: n.a. = not applicable;
n.p. = not provided