

## AR TA Assays Using Human Cells

Reference	Wang and Fondell (2001)	Wang and Fondell (2001)
<b>Characteristics of Cell Line</b>		
Name of cell line	HeLa (E19)	HeLa (E19)
Cell source	Human cervical tumor	Human cervical tumor
<b>Transfection of Cells with Plasmids</b>		
Stable or transient transfection	Stable AR/Transient reporter	Stable AR/Transient reporter
AR expression vector	pTetCMV-F0(S)-AR	pTetCMV-F0(S)-AR
AR source	human	human
Reporter vector	MMTV-Luc	ARE2-DS-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	none
Endpoint measured for cell toxicity	n.a.	n.a.
<b>Preparation of Cells for Assay</b>		
<i>Transient</i>		
Pregrowth of cells before transient transfection	One day prior to transfection with reporter vector	One day prior to transfection with reporter vector
Time from transient transfection to treatment of cells	3 hours	3 hours
<i>Stable</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
<b>Transcriptional Activation Assay</b>		
Test substance solvent	n.p.	n.p.
No. of replicates	n.p.	n.p.
No. of times assay repeated	3	3
Test substance incubation time	48 hours	48 hours
<b>Agonism assay</b>		
Reference ligand	Testosterone	Testosterone
Final concentration of reference ligand	100 nM	100 nM
<b>Antagonism assay</b>		
Reference ligand	Testosterone	Testosterone
Final concentration of reference ligand	n.p.	n.p.

Abbreviations: n.a. = not applicable;

n.p. = not provided

## AR TA Assays Using Human Cells

Reference	Wang and Fondell (2001)	Gaido et al. (2000)
<b>Characteristics of Cell Line</b>		
Name of cell line	HeLa (E19)	HepG2
Cell source	Human cervical tumor	Human hepatoma
<b>Transfection of Cells with Plasmids</b>		
Stable or transient transfection	Stable AR/Transient reporter	Transient AR/Transient reporter
AR expression vector	pTetCMV-F0(S)-AR	n.p.
AR source	human	human
Reporter vector	PB(-285/+32)-Luc	MMTV-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	pCMV -gal
Endpoint measured for cell toxicity	n.a.	-galactosidase activity
<b>Preparation of Cells for Assay</b>		
<i>Transient</i>		
Pregrowth of cells before transient transfection	One day prior to transfection with reporter vector	overnight
Time from transient transfection to treatment of cells	3 hours	n.p.
<i>Stable</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
<b>Transcriptional Activation Assay</b>		
Test substance solvent	n.p.	n.p.
No. of replicates	n.p.	3
No. of times assay repeated	3	At least 3
Test substance incubation time	48 hours	24 hours
<b>Agonism assay</b>		
Reference ligand	Testosterone	n.a.
Final concentration of reference ligand	100 nM	n.a.
<b>Antagonism assay</b>		
Reference ligand	Testosterone	5 $\alpha$ -Dihydrotestosterone
Final concentration of reference ligand	n.p.	n.p.

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## AR TA Assays Using Human Cells

Reference	Maness et al. (1998)	Tamura et al. (2001)
<b>Characteristics of Cell Line</b>		
Name of cell line	HepG2	HepG2
Cell source	Human hepatoma	Human hepatoma
<b>Transfection of Cells with Plasmids</b>		
Stable or transient transfection	Transient AR/Transient reporter	Transient AR/Transient reporter
AR expression vector	pRSAR	pRSAR
AR source	human	human
Reporter vector	MMTV-Luc	MMTV-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	pCMV -gal	pCMV -gal
Endpoint measured for cell toxicity	-galactosidase activity	-galactosidase activity
<b>Preparation of Cells for Assay</b>		
<i>Transient</i>		
Pregrowth of cells before transient transfection	overnight	n.p.
Time from transient transfection to treatment of cells	3 hours	n.p.
<i>Stable</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
<b>Transcriptional Activation Assay</b>		
Test substance solvent	DMSO	DMSO
No. of replicates	3	3
No. of times assay repeated	3 to 9	3
Test substance incubation time	24 hours	24 hours
<b>Agonism assay</b>		
Reference ligand	5 -Dihydrotestosterone	n.a.
Final concentration of reference ligand	1 $\mu$ M	n.a.
<b>Antagonism assay</b>		
Reference ligand	5 -Dihydrotestosterone	5 -Dihydrotestosterone
Final concentration of reference ligand	n.p.	n.p.

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## AR TA Assays Using Human Cells

Reference	Wilson et al. (2002)	Hartig et al. (2002)
<b>Characteristics of Cell Line</b>		
Name of cell line	MDA-kb2	MDA-MB-453
Cell source	Derived from MDA-MB-453 cells (human breast carcinoma)	Human breast carcinoma
<b>Transfection of Cells with Plasmids</b>		
Stable or transient transfection	Stable reporter	Transduced reporter
AR expression vector	Endogenous hAR	Endogenous hAR
AR source	human	human
Reporter vector	pMMTV.Luc.neo	Ad/mLuc7
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	none
Endpoint measured for cell toxicity	n.a.	n.a.
<b>Preparation of Cells for Assay</b>		
<i>Transient</i>		
Pregrowth of cells before transient transfection	n.a.	24 hours
Time from transient transfection to treatment of cells	n.a.	4 hours
<i>Stable</i>		
Plating time prior to treatment with test substance	4 to 6 hours	n.a.
<b>Transcriptional Activation Assay</b>		
Test substance solvent	Ethanol	Ethanol
No. of replicates	At least 4	3 to 4
No. of times assay repeated	3	n.p.
Test substance incubation time	overnight	48 hours
<b>Agonism assay</b>		
Reference ligand	n.a.	5 $\alpha$ -Dihydrotestosterone
Final concentration of reference ligand	n.a.	0.1 nM
<b>Antagonism assay</b>		
Reference ligand	5 $\alpha$ -Dihydrotestosterone	5 $\alpha$ -Dihydrotestosterone
Final concentration of reference ligand	0.1 or 1.0 nM	0.1 nM

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n.p. = not provided

## AR TA Assays Using Human Cells

Reference	Lambright et al. (2000)	Sultan et al. (2001)
<b>Characteristics of Cell Line</b>		
Name of cell line	MDA-MB-453-kb2	PALM
Cell source	Human breast carcinoma	Derived from PC-3 cells (human prostate adenocarcinoma)
<b>Transfection of Cells with Plasmids</b>		
Stable or transient transfection	Stable reporter	Stable AR/Stable reporter
AR expression vector	Endogenous hAR	pSG5-puro-hAR
AR source	human	human
Reporter vector	MMTV.neo.luciferase	pMMTV-neo-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	pCMV -gal	none
Endpoint measured for cell toxicity	-galactosidase activity	n.a.
<b>Preparation of Cells for Assay</b>		
<i>Transient</i>		
Pregrowth of cells before transient transfection	n.a.	n.a.
Time from transient transfection to treatment of cells	n.a.	n.a.
<i>Stable</i>		
Plating time prior to treatment with test substance	5 to 6 hours	n.p.
<b>Transcriptional Activation Assay</b>		
Test substance solvent	Ethanol	n.p.
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Test substance incubation time	overnight	n.p.
<b>Agonism assay</b>		
Reference ligand	n.a.	n.a.
Final concentration of reference ligand	n.a.	n.a.
<b>Antagonism assay</b>		
Reference ligand	5 $\alpha$ -Dihydrotestosterone	Methyltrienolone
Final concentration of reference ligand	0.1 nM	0.1 nM

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## AR TA Assays Using Human Cells

Reference	Terouanne et al. (2000)	Terouanne et al. (2000)
<b>Characteristics of Cell Line</b>		
Name of cell line	PALM	PC-3
Cell source	Derived from PC-3 cells (human prostate adenocarcinoma)	Human prostate adenocarcinoma
<b>Transfection of Cells with Plasmids</b>		
Stable or transient transfection	Stable AR/Stable reporter	Transient AR/Transient reporter
AR expression vector	pSG <sub>5</sub> -puro-hAR	pSG <sub>5</sub> -puro-hAR
AR source	human	human
Reporter vector	pMMTV-neo-Luc	pMAMneo-Luc
Endpoint measured	Luciferase activity	Luciferase activity
Plasmid transfected for cell toxicity	none	pCMV <sub>5</sub> -galactosidase
Endpoint measured for cell toxicity	n.a.	-galactosidase activity
<b>Preparation of Cells for Assay</b>		
<i>Transient</i>		
Pregrowth of cells before transient transfection	n.a.	n.p.
Time from transient transfection to treatment of cells	n.a.	n.p.
<i>Stable</i>		
Plating time prior to treatment with test substance	12 hours	n.a.
<b>Transcriptional Activation Assay</b>		
Test substance solvent	F12 medium	n.p.
No. of replicates	2	2
No. of times assay repeated	At least 3	At least 3
Test substance incubation time	30 hours	n.p.
<b>Agonism assay</b>		
Reference ligand	Methyltrienolone	n.a.
Final concentration of reference ligand	0.1 nM	n.a.
<b>Antagonism assay</b>		
Reference ligand	Methyltrienolone	Methyltrienolone
Final concentration of reference ligand	0.1 nM	0.1 nM

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## AR TA Assays Using Human Cells

Reference	Schrader and Cooke (2000)
<b>Characteristics of Cell Line</b>	
Name of cell line	PC-3 LUC <sup>AR+</sup>
Cell source	Derived from PC-3 cells (human prostate adenocarcinoma)
<b>Transfection of Cells with Plasmids</b>	
Stable or transient transfection	Stable AR/Stable reporter
AR expression vector	pCMV5-hAR
AR source	human
Reporter vector	MMTV.pMAMneo-Luc
Endpoint measured	Luciferase activity
Plasmid transfected for cell toxicity	none
Endpoint measured for cell toxicity	n.a.
<b>Preparation of Cells for Assay</b>	
<i>Transient</i>	
Pregrowth of cells before transient transfection	n.a.
Time from transient transfection to treatment of cells	n.a.
<i>Stable</i>	
Plating time prior to treatment with test substance	24 hours
<b>Transcriptional Activation Assay</b>	
Test substance solvent	DMSO
No. of replicates	n.p.
No. of times assay repeated	n.p.
Test substance incubation time	18 hours
<b>Agonism assay</b>	
Reference ligand	5 $\alpha$ -Dihydrotestosterone
Final concentration of reference ligand	n.p.
<b>Antagonism assay</b>	
Reference ligand	5 $\alpha$ -Dihydrotestosterone
Final concentration of reference ligand	n.p.

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