

ER TA Reporter Gene Assays Using MCF-7 Cells

	Bonfeld-Jørgensen et al. (2001)	Charles et al. (2000a)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	n.p.	Lipofectin
ER expression vector	endogenous	GAL4-HEG0 (chimeric hER def)
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	pERE-tk-CAT	17 m5-G-Luc
Reporter/endpoint	CAT	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pON249 (-gal)	pCH110 (-gal)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	6 hours
Time from transient transfection to treatment of cells	n.p.	16-18 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	yes
Metabolic activation source	n.a.	S9 from Aroclor-induced male Sprague Dawley rat
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	3 to 9 μ M or 0.1 nM	1 pM to 10 μ M
No. of replicates	3	n.p.
No. of times assay repeated	3 to 4	n.p.
Agonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	10 nM	1 pM - 10 nM
Incubation time of test substance	48 hours	24 hours
Antagonism assay		
Reference ligand	17 -Estradiol	not done
Final concentration of reference ligand	10 nM	not done
Incubation time of test substance	not done	not done

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Charles et al. (2000b)	Clemons et al. (1998)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Lipofectin	Calcium phosphate
ER expression vector	GAL4-HEG0 (chimeric hER def)	GAL4-HEG0 (chimeric hER def)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	17 m5-G-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCH110 (-gal)	pCMV (-gal)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	6 hours	6 hours
Time from transient transfection to treatment of cells	16-18 hours	Overnight
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	10 nM to 10 μ M	10 pM to 0.1 μ M
No. of replicates	3	2
No. of times assay repeated	3 to 5	2
Agonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	1 pM - 10 nM	n.p.
Incubation time of test substance	24 hours	24 hours
Antagonism assay		
Reference ligand	not done	17 -Estradiol
Final concentration of reference ligand	not done	n.p.
Incubation time of test substance	not done	not done

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ER TA Reporter Gene Assays Using MCF-7 Cells

	Collins-Burow et al. (2000)	Connor et al. (1996)
Characteristics of Cell Line		
Cell subtype	M variant p250	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Lipofect AMINE™	Calcium phosphate
ER expression vector	endogenous	GAL4-HEG0 (chimeric hER def)
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	pERE2-luciferase (vitellogenin ERE linked to Luc)	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV (-gal)	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	Overnight	n.p.
Time from transient transfection to treatment of cells	n.p.	24 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO
Range of test substance concentrations	1, 10, 25 µM	1 nM to 10 µM
No. of replicates	3	n.p.
No. of times assay repeated	3	At least 3
Agonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	1 nM	0.1 pM to 10 nM
Incubation time of test substance	18 - 24 hours	24 hours
Antagonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	1 nM	1 nM
Incubation time of test substance	18 - 24 hours	24 hours

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ER TA Reporter Gene Assays Using MCF-7 Cells

	Connor et al. (1997)	Fertuck et al. (2001a)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	n.p.	Calcium phosphate
ER expression vector	hER (undefined)	GAL4-HEG0 (chimeric hER def)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	Vit-CAT plasmid	17m5-G-Luc
Reporter/endpoint	CAT	luciferase
Reporter transfection	Transient	Transient
Other plasmids	none	pCMV-lacZ
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	6 hours
Time from transient transfection to treatment of cells	12 hours	18 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	10 μ M	0.1 nM to 10 μ M
No. of replicates	n.p.	n.p.
No. of times assay repeated	At least 3	At least 3
Agonism assay		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Final concentration of reference ligand	1 nM	10 nM
Incubation time of test substance	48 hours	24 hours
Antagonism assay		
Reference ligand	17 β -Estradiol	
Final concentration of reference ligand	1 nM	
Incubation time of test substance		not done

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	Fertuck et al. (2001a)	Fertuck et al. (2001b)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	Calcium phosphate
ER expression vector	GAL4-mER def	GAL4-HEG0 (chimeric hER def)
ER source	mouse	human
ER transfection	Transient	Transient
Reporter vector	17m5-G-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-lacZ	pCMV-lacZ
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	6 hours	n.p.
Time from transient transfection to treatment of cells	18 hours	18 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	0.1 nM to 10 μ M	0.1 to 10 μ M
No. of replicates	n.p.	n.p.
No. of times assay repeated	At least 3	At least 3
<i>Agonism assay</i>		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Final concentration of reference ligand	10 nM	1 nM
Incubation time of test substance	24 hours	24 hours
<i>Antagonism assay</i>		
Reference ligand		17 β -Estradiol
Final concentration of reference ligand		0.1 and 1 nM
Incubation time of test substance		24 hours

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ER TA Reporter Gene Assays Using MCF-7 Cells

	Fertuck et al. (2001b)	Fielden et al. (1997)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	Calcium phosphate
ER expression vector	GAL4-mER def	GAL4-HEG0 (chimeric hER def)
ER source	mouse	human
ER transfection	Transient	Transient
Reporter vector	17m5-G-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV-lacZ	pCMV-lacZ
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	6 hours
Time from transient transfection to treatment of cells	18 hours	16 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	0.1 to 10 μ M	0.1 to 10 μ M
No. of replicates	n.p.	2
No. of times assay repeated	At least 3	3
Agonism assay		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Final concentration of reference ligand	1 nM	5 nM
Incubation time of test substance	24 hours	24 hours
Antagonism assay		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Final concentration of reference ligand	0.1 and 1 nM	1 nM
Incubation time of test substance	24 hours	24 hours

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ER TA Reporter Gene Assays Using MCF-7 Cells

	Jobling et al. (1995)	Klotz et al. (1996)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	Lipofect AMINE™
ER expression vector	endogenous	endogenous
ER source	human	human
ER transfection	n.a.	n.a.
Reporter vector	pERE-tk-Luc (vitellogenin A2)	pERE2-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pJ7LacZ	pCMV (-gal)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	to 80% confluence	72 hours
Time from transient transfection to treatment of cells	n.p.	5 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO or ethanol
Range of test substance concentrations	0.1 to 10 µM	1 pM to 20 µM
No. of replicates	2	3
No. of times assay repeated	2	At least 2
Agonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	10 nM	0.1 nM
Incubation time of test substance	24 hours	18 hours
Antagonism assay		
Reference ligand	17 -Estradiol	
Final concentration of reference ligand	10 pM	
Incubation time of test substance	24 hours	

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

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	Klotz et al. (1997)	Kramer et al. (1997)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Lipofect AMINE™	n.p.
ER expression vector	endogenous	endogenous
ER source	human	human
ER transfection	n.a.	n.a.
Reporter vector	pERE2-Luc	pVit-tk-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids	pCMV (-gal)	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	72 hours	n.a.
Time from transient transfection to treatment of cells	5 hours	n.a.
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	48 hours
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or ethanol	Ethanol
Range of test substance concentrations	100 nM	0.1 to 10 µM
No. of replicates	3	4
No. of times assay repeated	2	n.p.
Agonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	0.5 nM	0.1 nM
Incubation time of test substance	18 hours	48 hours
Antagonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	0.5 nM	0.1 nM
Incubation time of test substance	18 hours	48 hours

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Lascombe et al. (2000)	Matthews et al. (2001)
Characteristics of Cell Line		
Cell subtype	MELN41	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	n.p.	Calcium phosphate
ER expression vector	endogenous	Gal4-hER def
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	ERE-Luc	17m-5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	none	pCMV-lacZ
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.a.	7 hours
Time from transient transfection to treatment of cells	n.a.	16 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	24 hours	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	100 nM to 100 μ M	1 pM to 10 μ M
No. of replicates	2	n.p.
No. of times assay repeated	3	2 to 3
Agonism assay		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Final concentration of reference ligand	10 nM	1 pM - 10 nM
Incubation time of test substance	12 hours	24 hours
Antagonism assay		
Reference ligand	17 β -Estradiol	
Final concentration of reference ligand	10 nM	
Incubation time of test substance	12 hours	not done

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Matthews et al. (2001)	Ramamoorthy et al. (1997a)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Calcium phosphate	n.p.
ER expression vector	Gal4-hER def	hER (undefined)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	17m-5-G-Luc	CKB-CAT (rat creatine kinase B construct) or CATH-CAT (human cathepsin)
Reporter/endpoint	luciferase	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCMV-lacZ	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	7 hours	n.p.
Time from transient transfection to treatment of cells	16 hours	14 - 16 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	1 pM to 10 μ M	10 nM to 10 μ M
No. of replicates	n.p.	n.p.
No. of times assay repeated	2 to 3	3
Agonism assay		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Final concentration of reference ligand	1 pM - 10 nM	10 nM
Incubation time of test substance	24 hours	14-16 hours
Antagonism assay		
Reference ligand		
Final concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Ramamoorthy et al. (1997b)	Sumida et al. (2001)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	"transfection cocktail"	Lipofectin
ER expression vector	hER (undefined)	endogenous
ER source	human	human
ER transfection	Transient	n.a.
Reporter vector	CKB-CAT or CATH-CAT	pGV-tk-vEREx5
Reporter/endpoint	CAT	luciferase
Reporter transfection	Transient	Transient
Other plasmids	none	none
Other plasmid transfection	n.a.	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	14 - 16 hours	Overnight
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	yes
Metabolic activation source	n.a.	S9 from induced rat liver
Test substance solvent	n.p.	DMSO
Range of test substance concentrations	10 nM to 10 μ M	n.p.
No. of replicates	n.p.	4
No. of times assay repeated	3	n.p.
<i>Agonism assay</i>		
Reference ligand	17 -Estradiol	none
Final concentration of reference ligand	10 nM	n.p.
Incubation time of test substance	48 hours	24-28 hours
<i>Antagonism assay</i>		
Reference ligand		
Final concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.

ER TA Reporter Gene Assays Using MCF-7 Cells

	Yoshihara et al. (2001)	Zacharewski et al. (1998)
Characteristics of Cell Line		
Cell subtype	n.p.	n.p.
Cell source	human breast cancer	human breast cancer
Transfection of Cells with Plasmids		
Transfection reagent	Transfast™	Calcium phosphate
ER expression vector	endogenous	GAL4-HEG0 (chimeric hER def)
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	p(ERE)3-SV40-Luc	17m5-G-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pRL/CMV	pCMV-lacZ
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	n.p.
Time from transient transfection to treatment of cells	n.p.	24 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	n.a.
Transcriptional Activation Assay		
Metabolic activation	yes	no
Metabolic activation source	S9 from male Wistar rat	n.a.
Test substance solvent	Ethanol	DMSO
Range of test substance concentrations	50 nM and 500 nM	100 nM to 10 µM
No. of replicates	n.p.	4
No. of times assay repeated	4	n.p.
Agonism assay		
Reference ligand	17 -Estradiol	17 -Estradiol
Final concentration of reference ligand	0.1 nM	10 nM
Incubation time of test substance	24 hours	24 hours
Antagonism assay		
Reference ligand	not done	not done
Final concentration of reference ligand		
Incubation time of test substance		

Abbreviations: DMSO = dimethyl sulfoxide;
n.a. = not applicable; n.p. = not provided.