

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Balaguer et al. (1996)	Bonfeld-Jørgensen et al. (2001)
Characteristics of Cell Line		
Cell line	HeLa	MDA-MB-231
Cell subtype		
Cell source	human cervical cancer	human breast cancer (ER-neg)
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate precipitation	n.p.
ER expression vector	Gal4-HEGO (hER def)	pSG5-HEO (ER)
ER source	human	human
ER transfection	Stable	Transient
Reporter vector	p17m5-G-Luc	pERE-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	pAG60 (neo)	none
Other plasmid transfection	Stable	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</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 transfection</i>		
Plating time prior to treatment with test substance	16 hours	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	Ethanol
No. of replicates	2	At least 9
No. of times assay repeated	At least 3	3 or 4
Range of test substance concentrations	100 nM to 10 μ M	0.1 μ M or 9 μ M
<i>Agonism</i>		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	100 nM to 10 μ M	10 nM
Incubation time of test substance	24 hours	n.p.
<i>Antagonism</i>		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	1 nM	10 nM
Incubation time of test substance	24 hours	n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Connor et al. (1997)	Gaido et al. (1999)
Characteristics of Cell Line		
Cell line	HeLa	HeLa
Cell subtype		
Cell source	human cervical cancer	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Superfect
ER expression vector	Gal4-HEGO (hER def)	hER (undefined)
ER source	human	human
ER transfection	Stable	Transient
Reporter vector	17m5-G-Luc	vtERE-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	none	pCMV- -gal
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
<i>Transient transfection</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 transfection</i>		
Plating time prior to treatment with test substance	16 hours	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. of replicates	4	3
No. of times assay repeated	At least 3	3 to 4
Range of test substance concentrations	100 nM to 10 μ M	1 pM to 1 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	1 nM	1 pM to 100 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	24 hours	24 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (1999)	Gaido et al. (1999)
Characteristics of Cell Line		
Cell line	HeLa	HepG2
Cell subtype		
Cell source	human cervical cancer	human liver tumor
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfect	Superfect
ER expression vector	hER (undefined)	hER (undefined)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	vtERE-Luc	C3-Luc or MMTV-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV- -gal	pCMV- -gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	Overnight
Time from transient transfection to treatment of cells	n.p.	n.p.
<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
No. of replicates	3	3
No. of times assay repeated	3 to 4	3 or 4
Range of test substance concentrations	1 pM to 1 μ M	0.1 nM to 10 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	1 pM to 100 nM	10 pM to 100 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours

Abbreviations: DMSO = dimethyl sulfoxide;
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ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (1999)	Gaido et al. (1999)
Characteristics of Cell Line		
Cell line	HepG2	HepG2
Cell subtype		
Cell source	human liver tumor	human liver tumor
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfect	Superfect
ER expression vector	hER (undefined)	rER (undefined)
ER source	human	rat
ER transfection	Transient	Transient
Reporter vector	C3-Luc or MMTV-Luc	C3-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV- -gal	pCMV- -gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	Overnight	Overnight
Time from transient transfection to treatment of cells	n.p.	n.p.
<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
No. of replicates	3	3
No. of times assay repeated	3 or 4	3 or 4
Range of test substance concentrations	0.1 nM to 10 µM	0.1 nM to 10 µM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	10 pM to 100 nM	10 pM to 100 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (1999)	Gaido et al. (2000)
Characteristics of Cell Line		
Cell line	HepG2	HepG2
Cell subtype		
Cell source	human liver tumor	human liver tumor
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfect	Superfect
ER expression vector	rER (undefined)	hER (undefined)
ER source	rat	human
ER transfection	Transient	Transient
Reporter vector	C3-Luc	C3-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCMV- -gal	pCMV- -gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	Overnight	Overnight
Time from transient transfection to treatment of cells	n.p.	n.p.
<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
No. of replicates	3	n.p.
No. of times assay repeated	3 or 4	3
Range of test substance concentrations	0.1 nM to 10 µM	10 nM to 10 µM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	10 pM to 100 nM	0.6 µM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	n.p.	0.6 µM
Incubation time of test substance	24 hours	n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gaido et al. (2000)	Garner et al. (1999)
Characteristics of Cell Line		
Cell line	HepG2	HeLa
Cell subtype		
Cell source	human liver tumor	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Superfect	Electroporation
ER expression vector	hER (undefined)	pRSV
ER source	human	mouse
ER transfection	Transient	Transient
Reporter vector	C3-Luc	ERET81CAT
Reporter/endpoint	luciferase	CAT
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.	n.p.
<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 or Ethanol
No. of replicates	n.p.	n.p.
No. of times assay repeated	3	3
Range of test substance concentrations	10 nM to 10 μ M	1 nM to 10 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	0.6 μ M	10 nM
Incubation time of test substance	24 hours	28 hours
Antagonism		
Reference ligand	17 β -Estradiol	
Concentration of reference ligand	0.6 μ M	
Incubation time of test substance	n.p.	

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Gould et al. (1998)	Hodges et al. (2000)
Characteristics of Cell Line		
Cell line	HepG2	ELT-3
Cell subtype		
Cell source	human liver tumor	rat uterine leiomyoma
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Calcium phosphate
ER expression vector	hER (undefined)	pRSVT7
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	pC3-Luc	ERE-tk-LUC6a
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pRSV- -gal	pCMV- -gal
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	18 hours	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	DMSO or methanol	DMSO
No. of replicates	3	3
No. of times assay repeated	4 to 6	n.p.
Range of test substance concentrations	10 pM to 10 μ M	100 nM to 100 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand		10 nM
Incubation time of test substance	24 hours	48 hours
Antagonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand		n.p.
Incubation time of test substance	24 hours	n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Hoogenboom et al. (2001)	Klotz et al. (1997)
Characteristics of Cell Line		
Cell line	T47D	Ishikawa
Cell subtype		
Cell source	human breast adenocarcinoma	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Lipofect AMINE™
ER expression vector	endogenous	pSG5-hER
ER source	human	human
ER transfection	n.a.	Transient
Reporter vector	p-EREtata-Luc	pERE2luc
Reporter/endpoint	luciferase	luciferase induction
Reporter transfection	Stable	Transient
Other plasmids	none	pCMV- -gal
Other plasmid transfection	n.a.	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.a.	72 hours
Time from transient transfection to treatment of cells	n.a.	5 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.p.	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO or ethanol
No. of replicates	3	3
No. of times assay repeated	n.p.	2
Range of test substance concentrations	.0001 to 10,000 nM	100 nM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	varied	1 nM
Incubation time of test substance	24 hours	18 hours
Antagonism		
Reference ligand		17 -Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		18 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Kraichely et al. (2000)	Kraichely et al. (2000)
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pBD-GAL4 (ER)	pBD-GAL4 (ER)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	(ERE)3-pS2-CAT	(ERE)3-pS2-CAT
Reporter/endpoint	CAT expression	CAT expression
Reporter transfection	Transient	Transient
Other plasmids	pCMV- -gal	pCMV- -gal
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.	n.p.
<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.
No. of replicates	n.p.	n.p.
No. of times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 pM to 1 μM	1 pM to 1 μM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Antagonism		
Reference ligand		17 -Estradiol
Concentration of reference ligand		n.p.
Incubation time of test substance		n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Kuiper et al. (1998)	Kuiper et al. (1998)
Characteristics of Cell Line		
Cell line	HEK 293	HEK 293
Cell subtype		
Cell source	human embryonal kidney	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pSG5-HEGO (hER def)	pSG5-hER
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	3xERE-ATAT-LUC	3xERE-ATAT-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	-gal (not described)	-gal (not described)
Other plasmid transfection	Transient	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	24 hours	24 hours
Time from transient transfection to treatment of cells	16 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	ethanol	ethanol
No. of replicates	3	3
No. of times assay repeated	2	2
Range of test substance concentrations	1 nM to 1 μ M	1 nM to 1 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	18 hours	18 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Legler et al. (1999)	Makela et al. (1994)
Characteristics of Cell Line		
Cell line	T47D	HeLa
Cell subtype		
Cell source	human breast adenocarcinoma	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Electroporation
ER expression vector	endogenous	pRSV-ER
ER source	human	mouse
ER transfection	n.a.	Transient
Reporter vector	p-EREtata-Luc	ERET81CAT
Reporter/endpoint	luciferase	CAT expression
Reporter transfection	Stable	Transient
Other plasmids	pGK-Hyg	none
Other plasmid transfection	Stable	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.a.	n.p.
Time from transient transfection to treatment of cells	n.a.	4 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	48 hours	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or ethanol	n.p.
No. of replicates	3	3
No. of times assay repeated	n.p.	n.p.
Range of test substance concentrations	.01 to 1000 nM	100 nM
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	30 pM	1 nM
Incubation time of test substance	24 hours	28 hours
Antagonism		
Reference ligand	17 β -Estradiol	
Concentration of reference ligand	6 pM	
Incubation time of test substance	n.p.	

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Meerts et al. (2001)	Meerts et al. (2001)
Characteristics of Cell Line		
Cell line	T47D	HEK 293
Cell subtype		
Cell source	human breast adenocarcinoma	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	n.p.
ER expression vector	endogenous	pSG5-hER
ER source	human	human
ER transfection	n.a.	Stable
Reporter vector	pEREtata-Luc	3xERE-ATAT-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Stable
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.a.	n.a.
Time from transient transfection to treatment of cells	n.a.	n.a.
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	48 hours	48 hours
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO or ethanol	DMSO or ethanol
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Range of test substance concentrations	10 to 10,000 nM	100 to 10,000 nM
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand	17 β -Estradiol	
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.

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Meyers et al. (1999)	Meyers et al. (1999)
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	n.p.
ER expression vector	hER (plasmid unspecified)	hER (plasmid unspecified)
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	(ERE) ₃ -pS2-CAT	(ERE) ₃ -pS2-CAT
Reporter/endpoint	CAT	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCMV- -gal	pCMV- -gal
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.	n.p.
<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.
No. of replicates	n.p.	n.p.
No. of times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 nM to 1 μM	1 nM to 1 μM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	10 nM	10 nM
Incubation time of test substance	n.p.	n.p.
Antagonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	1 nM	1 nM
Incubation time of test substance	n.p.	n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Miksicek (1993)	Miksicek (1994)
Characteristics of Cell Line		
Cell line	HeLa	HeLa
Cell subtype		
Cell source	human cervical cancer	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pER-18	pER-18
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	pERE-TK-CAT	pERE-TK-CAT
Reporter/endpoint	CAT	CAT
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	24 hours	24 hours
Time from transient transfection to treatment of cells	5-6 hours	5-6 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	Ethanol	Ethanol
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Range of test substance concentrations	varies; 10 pM-10 nM & 1 nM-10 μM & 10 nM-10 μM	varies for each substance; from 1 pM to 10 μM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	5 nM	1 nM
Incubation time of test substance	48 hours	48 hours
Antagonism		
Reference ligand		17 -Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		48 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Moore et al. (1997)	Otsuka Pharmaceutical (2001)
Characteristics of Cell Line		
Cell line	HeLa	CHO
Cell subtype		K1
Cell source	human cervical cancer	Chinese hamster ovary
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	n.p.
ER expression vector	Gal4-HEGO (hER def)	pcDNA ER
ER source	human	n.p.
ER transfection	Stable	Transient
Reporter vector	p17m5-G-Luc	pGL3ERE-7
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	Transient
Other plasmids	pAG60 (neo)	pcDNA-EGFP
Other plasmid transfection	Stable	Transient
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.a.	24
Time from transient transfection to treatment of cells	n.a.	3
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	16 hours	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. of replicates	2	4
No. of times assay repeated	4	2
Range of test substance concentrations	10 nM to 10 μ M	varies; about 10 pM to 10 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	24 hours	16-24 hours
Antagonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	24 hours	n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Otsuka Pharmaceutical (2001)	Ramamoorthy et al. (1997b)
Characteristics of Cell Line		
Cell line	CHO	MDA-MB-231
Cell subtype	K1	
Cell source	Chinese hamster ovary	human breast cancer (ER-neg)
Transfection of Cells with Plasmids		
Transfection method/reagent	n.p.	Calcium phosphate
ER expression vector	pcDNA ER	hER (undefined)
ER source	n.p.	human
ER transfection	Stable	Transient
Reporter vector	pINDERE-15	pC3-Luc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Stable	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.a.	24 hours
Time from transient transfection to treatment of cells	n.a.	18 hours
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	24	n.a.
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	DMSO	DMSO
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Range of test substance concentrations	varies; about 1 pM to 100 nM	10 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	.001 μ M	10 nM
Incubation time of test substance	16-24 hours	2 days
Antagonism		
Reference ligand		
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 Various Mammalian Cells

	Ramamoorthy et al. (1997b)	Rogers and Denison (2000)
Characteristics of Cell Line		
Cell line	HepG2	BG-1
Cell subtype		BG1Luc4E2
Cell source	human liver cancer	human ovarian carcinoma
Transfection of Cells with Plasmids		
Transfection method/reagent	Lipofectin and OptiMEM	Polyprene
ER expression vector	hER (undefined)	endogenous
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	pC3-LUC	pGudLuc7ere
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	-gal plasmid	none
Other plasmid transfection	Transient	n.a.
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	18 hours	n.p.
Time from transient transfection to treatment of cells	3 hours	48 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 or ethanol
No. of replicates	3	n.p.
No. of times assay repeated	n.p.	At least 3
Range of test substance concentrations	1 nM to 1 μ M	
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	n.p.	1 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand		17 β -Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		24 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Seinen et al. (1999)	Seinen et al. (1999)
Characteristics of Cell Line		
Cell line	HEK 293	HEK 293
Cell subtype		
Cell source	human embryonal kidney	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pSG5-HEGO (hER)	pSG5-HEGO (hER)
ER source	human	human
ER transfection	Transient	Stable
Reporter vector	3xERE-TATA-LUC	3xERE-TATA-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids	SV2-LacZ	SV2-LacZ
Other plasmid transfection	Transient	Stable
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	24 hours	n.a.
Time from transient transfection to treatment of cells	16 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	Ethanol	Ethanol
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Range of test substance concentrations	100 nM to 50 μ M	100 nM to 50 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	0.1 pM to 1 nM	1 pM to 1 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand		
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 Various Mammalian Cells

	Seinen et al. (1999)	Seinen et al. (1999)
Characteristics of Cell Line		
Cell line	HEK 293	HEK 293
Cell subtype		
Cell source	human embryonal kidney	human embryonal kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pSG5-hER	pSG5-hER
ER source	human	human
ER transfection	Transient	Stable
Reporter vector	3xERE-TATA-LUC	3xERE-TATA-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids	SV2-LacZ	SV2-LacZ
Other plasmid transfection	Transient	Stable
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	24 hours	n.a.
Time from transient transfection to treatment of cells	16 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	Ethanol	Ethanol
No. of replicates	n.p.	n.p.
No. of times assay repeated	n.p.	n.p.
Range of test substance concentrations	100 nM to 50 μ M	100 nM to 50 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	0.1 nM to 100 nM	10 pM to 10 nM
Incubation time of test substance	24 hours	24 hours
Antagonism		
Reference ligand		
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 Various Mammalian Cells

	Shelby et al. (1996)	Sumida et al. (2001)
Characteristics of Cell Line		
Cell line	HeLa	HeLa
Cell subtype		
Cell source	human cervical cancer	human cervical cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Electroporation	Lipofect AMINE™
ER expression vector	pRSV	pRc/RSV-hER
ER source	mouse	human
ER transfection	Transient	Transient
Reporter vector	ERET81CAT	pGV-tk-vEREx5
Reporter/endpoint	CAT expression	luciferase
Reporter transfection	Transient	Transient
Other plasmids		
Other plasmid transfection		
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.	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
No. of replicates	3	n.p.
No. of times assay repeated	3	n.p.
Range of test substance concentrations	varies for substance; 0.01 to 10,000 nM	100 pM to 10 μM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	1 nM	n.p.
Incubation time of test substance	28 hours	24-28 hours
Antagonism		
Reference ligand		
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 Various Mammalian Cells

	Sun et al. (1999)	Sun et al. (1999)
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pCMV5-hER	pCMV5-hER
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	ERE3-pS2-CAT	ERE3-pS2-CAT
Reporter/endpoint	CAT	CAT
Reporter transfection	Transient	Transient
Other plasmids	pCH110 or pCMV- -gal	pCH110 or pCMV- -gal
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.	n.p.
<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.
No. of replicates	n.p.	n.p.
No. of times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 pM to 1 μ M	1 pM to 1 μ M
Agonism		
Reference ligand	17 β -Estradiol	17 β -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Antagonism		
Reference ligand		17 β -Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Sun et al. (1999)	Sun et al. (1999)
Characteristics of Cell Line		
Cell line	HEC-1	HEC-1
Cell subtype		
Cell source	human endometrial cancer	human endometrial cancer
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pCMV5-hER	pCMV5-hER
ER source	human	human
ER transfection	Transient	Transient
Reporter vector	C3-Ti-LUC	C3-Ti-LUC
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids	pCH110 or pCMV- gal	pCH110 or pCMV- gal
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.	n.p.
<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.
No. of replicates	n.p.	n.p.
No. of times assay repeated	At least 3	At least 3
Range of test substance concentrations	1 pM to 1 μ M	1 pM to 1 μ M
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	n.p.	n.p.
Antagonism		
Reference ligand		17 -Estradiol
Concentration of reference ligand		1 nM
Incubation time of test substance		n.p.

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Tarumi et al. (2000)	Tremblay et al. (1998)
Characteristics of Cell Line		
Cell line	HeLa	COS-1
Cell subtype		
Cell source	human cervical cancer	monkey kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Lipofect AMINE™	Calcium phosphate
ER expression vector	hER (undefined)	pCMX-mER
ER source	human	mouse
ER transfection	Transient	Transient
Reporter vector	Luc (undefined)	vitA2-ERETKLuc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids		
Other plasmid transfection		
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	24 hours	n.p.
Time from transient transfection to treatment of cells	n.p.	8 to 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	n.p.
No. of replicates	6	n.p.
No. of times assay repeated	n.p.	3
Range of test substance concentrations	varies for substance; 0.1-10 µg/mL or 10 nmol/L - 1 µmol/L	100 nM or 10 pM to 100 nM
Agonism		
Reference ligand	none	17 -Estradiol
Concentration of reference ligand	n.a.	n.p.
Incubation time of test substance	24 hours	16 hours
Antagonism		
Reference ligand		17 -Estradiol
Concentration of reference ligand		10 nM
Incubation time of test substance		16 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Tremblay et al. (1998)	Tremblay et al. (1998)
Characteristics of Cell Line		
Cell line	COS-1	COS-1
Cell subtype		
Cell source	monkey kidney	monkey kidney
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	Calcium phosphate
ER expression vector	pCMX-mER	pCMX-mER
ER source	mouse	mouse
ER transfection	Transient	Transient
Reporter vector	vitA2-ERETKLuc	vitA2-EREBLuc
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Transient
Other plasmids		
Other plasmid transfection		
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	8 to 16 hours	8 to 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.
No. of replicates	n.p.	n.p.
No. of times assay repeated	3	3
Range of test substance concentrations	100 nM or 10 pM to 100 nM	100 nM or 10 pM to 100 nM
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	16 hours	16 hours
Antagonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	10 nM	10 nM
Incubation time of test substance	16 hours	16 hours

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Tremblay et al. (1998)	Xenobiotic Laboratory Systems, Inc.
Characteristics of Cell Line		
Cell line	COS-1	BG-1
Cell subtype		BG1Luc4E2
Cell source	monkey kidney	human ovarian carcinoma
Transfection of Cells with Plasmids		
Transfection method/reagent	Calcium phosphate	n.p.
ER expression vector	pCMX-mER	endogenous
ER source	mouse	human
ER transfection	Transient	n.a.
Reporter vector	vitA2-EREbLuc	pGudLuc7ere
Reporter/endpoint	luciferase	luciferase
Reporter transfection	Transient	Stable
Other plasmids		
Other plasmid transfection		
Preparation of Cells for Assay		
<i>Transient transfection</i>		
Pregrowth of cells before transient transfection	n.p.	n.a.
Time from transient transfection to treatment of cells	8 to 16 hours	n.a.
<i>Stable transfection</i>		
Plating time prior to treatment with test substance	n.a.	24 hours
Transcriptional Activation Assay		
Metabolic activation	no	no
Metabolic activation source	n.a.	n.a.
Test substance solvent	n.p.	DMSO
No. of replicates	n.p.	1
No. of times assay repeated	3	n.p.
Range of test substance concentrations	100 nM or 10 pM to 100 nM	10 µg/mL to 10 pg/mL
Agonism		
Reference ligand	17 -Estradiol	17 -Estradiol
Concentration of reference ligand	n.p.	n.p.
Incubation time of test substance	16 hours	24 hours
Antagonism		
Reference ligand	17 -Estradiol	
Concentration of reference ligand	10 nM	
Incubation time of test substance	16 hours	

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

ER TA Reporter Gene Assays Using Various Mammalian Cells

	Zacherewski et al. (1998)
Characteristics of Cell Line	
Cell line	HeLa
Cell subtype	
Cell source	human
Transfection of Cells with Plasmids	
Transfection method/reagent	Calcium phosphate
ER expression vector	Gal4-HEGO (hER def)
ER source	human
ER transfection	Stable
Reporter vector	17m5-G-Luc
Reporter/endpoint	luciferase
Reporter transfection	Stable
Other plasmids	
Other plasmid transfection	
Preparation of Cells for Assay	
<i>Transient transfection</i>	
Pregrowth of cells before transient transfection	n.a.
Time from transient transfection to treatment of cells	n.a.
<i>Stable transfection</i>	
Plating time prior to treatment with test substance	24 hours
Transcriptional Activation Assay	
Metabolic activation	no
Metabolic activation source	n.a.
Test substance solvent	DMSO
No. of replicates	2
No. of times assay repeated	3
Range of test substance concentrations	100 nM to 10 μ M
Agonism	
Reference ligand	17 β -Estradiol
Concentration of reference ligand	1 pM - 10 nM
Incubation time of test substance	24 hours
Antagonism	
Reference ligand	
Concentration of reference ligand	
Incubation time of test substance	

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