ER Cell Proliferation Assays

	Arcaro et al. (1998)	**Arcaro et al. (1999a,b)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	n.p.	n.p.
Cell Proliferation Assay		
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	maximum 5 µM	maximum 5 µM
No. of replicates	4	4
No. of times assay repeated	3	3
No. of cells/well	$1x10^5$cells/mL/well	$1x10^5$cells/mL/well
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	n.p.	1.0 nM
Cell division/incubation	14 days	14 days
Measured as (e.g., cell growth)	foci	foci
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	0.1 nM	1.0 nM
Cell division/incubation	14 days	14 days
Measured as (e.g., cell division)	foci	foci

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

ER Cell Proliferation Assays

	Bonefeld-Jørgensen et al. (2001)	**Collins-Burow et al. (2000)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7 (M variant p250)
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.001 to 10 µM	100 nM, 25 µM
No. of replicates	8	3
No. of times assay repeated	3	At least 2
No. of cells/well	10,000	50,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	10 pM	1.0 nM
Cell division/incubation	6 days	5 days
Measured as (e.g., cell growth)	cell growth	cell growth
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	10 pM	1.0 nM
Cell division/incubation	n.p.	5 days
Measured as (e.g., cell division)	cell growth	cell growth

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

ER Cell Proliferation Assays

	Dodge et al. (1996)	**Fielden et al. (1997)**
Characteristics of Cell Line		
Cell line	MCF-7 (ATCC HTB 22)	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	48 hours	n.p.
Cell Proliferation Assay		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0.001 to 1000 nM	0.01-10 µM
No. of replicates	3	3
No. of times assay repeated	n.p.	2
No. of cells/well	8000	n.p.
Agonism		
Reference ligand	17β-Estradiol	none
Final concentration of reference ligand	n.p.	n.a.
Cell division/incubation	48 hours	n.p.
Measured as (e.g., cell growth)	cell proliferation	cell growth
Antagonism	not done	
Reference ligand		17β-Estradiol
Final concentration of reference ligand		1 nM
Cell division/incubation		n.p.
Measured as (e.g., cell division)		cell growth

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

ER Cell Proliferation Assays

	Gierthy et al. (1997)	Go et al. (1999)
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	n.p.	Ethanol
Range of test substance concentrations	50 nM to 5 µM	0.001 to 100 µM
No. of replicates	4	2
No. of times assay repeated	n.p.	3
No. of cells/well	100,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	n.p.	10 nM
Cell division/incubation	14 days	6 days
Measured as (e.g., cell growth)	colony formation	cell proliferation
Antagonism		not done
Reference ligand	17β-Estradiol	
Final concentration of reference ligand	0.1 nM	
Cell division/incubation	14 days	
Measured as (e.g., cell division)	colony formation	

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

ER Cell Proliferation Assays

	Harris et al. (1997)	**Harris et al. (1997)**
Characteristics of Cell Line		
Cell line	MCF-7	ZR-75
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	3-4 days	n.p.
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	10 µM	10 nM to 10 µM
No. of replicates	2	2
No. of times assay repeated	2	2
No. of cells/well	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	10 nM	10 pM to 10 nM
Cell division/incubation	2, 5, 8, 12 days	11 days
Measured as (e.g., cell growth)	cell division	cell division
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Ichikawa et al. (1997)	**Jobling et al. (1995)**
Characteristics of Cell Line		
Cell line	MCF-7	ZR-75
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	n.p.	n.p.
Cell Proliferation Assay		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0.1 nM to 100 µM	10 µM
No. of replicates	n.p.	2
No. of times assay repeated	n.p.	2
No. of cells/well	2000	n.p.
Agonism		
Reference ligand	none	17β-Estradiol
Final concentration of reference ligand	n.a.	10 nM
Cell division/incubation	3 days	10 days
Measured as (e.g., cell growth)	cell growth	cell growth
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Jones et al. (1998)	**Korner et al. (1995)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	Overnight to attach + 48 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	up to 0.1 µM	up to 1 mM
No. of replicates	At least 3	At least 3
No. of times assay repeated	n.p.	4
No. of cells/well	10,000	10,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	1.0 nM	1 pM to 10 nM
Cell division/incubation	6 days	5 days
Measured as (e.g., cell growth)	cell proliferation	Total protein content (SRB assay) or mitochondrial metabolic activity (MTT) as estimates of cell number
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Le Guevel and Pakdel (2001)	**Makela et al. (1994)**
Characteristics of Cell Line		
Cell line	Ishikawa	MCF-7
Cell source	human endometrial tumor	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	About 1 day	2 days
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	varies by substance (about 10^{-12}-10^{-7} mol/L)	10 pM to 1 µM
No. of replicates	n.p.	n.p.
No. of times assay repeated	6	8 - 12
No. of cells/well	20,000 cells/200 µL	2000
Agonism		
Reference ligand	17α-Ethinyl estradiol	17β-Estradiol
Final concentration of reference ligand	10^{-13} to 10^{-10} mol/L	1 nM
Cell division/incubation	48 hours	7 days
Measured as (e.g., cell growth)	Alkaline phosphatase activity	cell growth
Antagonism	not done	
Reference ligand		17β-Estradiol
Final concentration of reference ligand		1 nM
Cell division/incubation		n.p.
Measured as (e.g., cell division)		cell growth

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

ER Cell Proliferation Assays

	Mellanen et al. (1996)	**Mellanen et al. (1996)**
Characteristics of Cell Line		
Cell line	MCF-7	T47D
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	About 1 day	About 1 day
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1.0 pM to 1 µM	1.0 fM to 1.0 µM
No. of replicates	3	3
No. of times assay repeated	n.p.	n.p.
No. of cells/well	2000	10,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	1 nM	1 nM
Cell division/incubation	7 days	10 days
Measured as (e.g., cell growth)	cell proliferation	cell proliferation
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Miksicek (1993)	**Miodini et al. (1999)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	n.p.	24 hours
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	n.p.	0.5 - 20 µM
No. of replicates	n.p.	4
No. of times assay repeated	n.p.	3
No. of cells/well	$5x10^3$ cell/cm^2	15,000
Agonism		
Reference ligand	17β-Estradiol	none
Final concentration of reference ligand	10 nM	
Cell division/incubation	1 week	6 days
Measured as (e.g., cell growth)	cell growth	cell growth
Antagonism	not done	
Reference ligand		17β-Estradiol
Final concentration of reference ligand		0.01 µM
Cell division/incubation		7 hours
Measured as (e.g., cell division)		cell growth

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

ER Cell Proliferation Assays

	Moore et al. (1997)	**Morito et al. (2001)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0 -10 µM	n.p.
No. of replicates	3	n.p.
No. of times assay repeated	n.p.	n.p.
No. of cells/well	50,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	1 nM	1 pM to 1 nM
Cell division/incubation	6 days	5 days
Measured as (e.g., cell growth)	cell growth	cell growth
Antagonism		not done
Reference ligand	17β-Estradiol	
Final concentration of reference ligand	1 nM	
Cell division/incubation	6 days	
Measured as (e.g., cell division)	cell growth	

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

ER Cell Proliferation Assays

	Nakagawa & Suzuki (2001)	Otsuka Pharmaceutical Co. (2001)
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	n.p.
Cell Proliferation Assay		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	1 nM to 500 µM	n.p.
No. of replicates	3 or 4	n.p.
No. of times assay repeated	n.p.	n.p.
No. of cells/well	4000	n.p.
Agonism		n.p.
Reference ligand	17β-Estradiol	n.p.
Final concentration of reference ligand	1 nM	n.p.
Cell division/incubation	5 days	n.p.
Measured as (e.g., cell growth)	cell proliferation	cell proliferation
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Payne et al. (2001)	**Ramamoorthy et al. (1997a)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours after seeding + 72 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.1 to 10 µM	100 nM to 10 µM
No. of replicates	3	3
No. of times assay repeated	2	n.p.
No. of cells/well	10,000	50,000
Agonism		
Reference ligand	none	17β-Estradiol
Final concentration of reference ligand	n.a.	1 nM
Cell division/incubation	7 days	11 days
Measured as (e.g., cell growth)	cell proliferation	cell growth
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Schafer et al. (1999)	**Schafer et al. (1999)**
Characteristics of Cell Line		
Cell line	MCF-7 (subline BUS)	T47D
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	Overnight to 3 days	Overnight to 3 days
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1 nM to 5 µM	1 nM to 5 µM
No. of replicates	Higher doses: 4	Higher doses: 4
No. of times assay repeated	n.p.	n.p.
No. of cells/well	10,000	10,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	1 nM	1 nM
Cell division/incubation	6 to 7 days	6 to 7 days
Measured as (e.g., cell growth)	cell proliferation	cell proliferation
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Schafer et al. (1999)	**Schlumpf et al. (2001)**
Characteristics of Cell Line		
Cell line	ZR-75-1	MCF-7 (Bos)
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	Overnight to 3 days	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1 nM to 5 µM	100 nM to 100 µM
No. of replicates	Higher doses: 4	4
No. of times assay repeated	n.p.	4 to 13
No. of cells/well	10,000	40,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	1 nM	0.1 pM to 10 nM
Cell division/incubation	6 to 7 days	6 days
Measured as (e.g., cell growth)	cell proliferation	cell proliferation
Antagonism	not done	
Reference ligand		17β-Estradiol
Final concentration of reference ligand		10 pM
Cell division/incubation		6 days
Measured as (e.g., cell division)		cell proliferation

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

ER Cell Proliferation Assays

	Soto et al. (1994)	**Soto et al. (1995)**
Characteristics of Cell Line		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	24 hours	24 hours
Cell Proliferation Assay		
Test substance solvent	Ethanol or DMSO	n.p.
Range of test substance concentrations	1 nM, 10 µM	100 nM to50 uM
No. of replicates	2	2
No. of times assay repeated	At least 5	At least 5
No. of cells/well	20,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	1.0 pM to 10 nM	10 or 30 pM
Cell division/incubation	6 days	6 days
Measured as (e.g., cell growth)	cell proliferation (relative proliferative potency & relative proliferative effect)	cell proliferation (relative proliferative potency & relative proliferative effect)
Antagonism	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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

ER Cell Proliferation Assays

	Tamir et al. (2000)	**Tamir et al. (2000)**
Characteristics of Cell Line		
Cell line	T47D	MCF-7
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	48 hours	n.p.
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.1 nM to 25 µM	1, 10, 25 µM
No. of replicates	n.p.	n.p.
No. of times assay repeated	3 or more	n.p.
No. of cells/well	n.p.	n.p.
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	100 pM	10 nM
Cell division/incubation	7 days	3 weeks
Measured as (e.g., cell growth)	cell proliferation	colony formation
Antagonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	100 pM	1 nM and 10 nM
Cell division/incubation	7 days	3 weeks
Measured as (e.g., cell division)	cell proliferation	colony formation

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

ER Cell Proliferation Assays

	Vinggaard et al. (1999)	**Wiese et al. (1997)**
Characteristics of Cell Line		
Cell line	MCF-7 (E3 clone)	MCF-7 (E3 clone)
Cell source	human breast cancer	human breast cancer
Preparation of Cells for Assay		
Plating time prior to treatment with test substance	5 days	n.p.
Cell Proliferation Assay		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.001, 0.1, 1, and 10 µM	1 pM to 1 µM
No. of replicates	At least 3	3
No. of times assay repeated	1, 2, 3, 4, 5, or 8 times	n.p.
No. of cells/well	15,000	20,000
Agonism		
Reference ligand	17β-Estradiol	17β-Estradiol
Final concentration of reference ligand	0.01 nM	10 pM
Cell division/incubation	up to 9 days	6 days
Measured as (e.g., cell growth)	cell proliferation	cell growth
Antagonism		not done
Reference ligand	17β-Estradiol	
Final concentration of reference ligand	0.01 nM	
Cell division/incubation	n.p.	
Measured as (e.g., cell division)	cell proliferation	

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