

## ER Cell Proliferation Assays

	Arcaro et al. (1998)	Arcaro et al. (1999a,b)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	n.p.	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	DMSO	DMSO
Range of test substance concentrations	maximum 5 $\mu$ M	maximum 5 $\mu$ M
No. of replicates	4	4
No. of times assay repeated	3	3
No. of cells/well	$1 \times 10^5$ cells/mL/well	$1 \times 10^5$ cells/mL/well
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7 (M variant p250)
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	24 hours	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.001 to 10 $\mu$ M	100 nM, 25 $\mu$ M
No. of replicates	8	3
No. of times assay repeated	3	At least 2
No. of cells/well	10,000	50,000
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7 (ATCC HTB 22)	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	48 hours	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0.001 to 1000 nM	0.01-10 $\mu$ M
No. of replicates	3	3
No. of times assay repeated	n.p.	2
No. of cells/well	8000	n.p.
<i>Agonism</i>		
Reference ligand	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	24 hours	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	n.p.	Ethanol
Range of test substance concentrations	50 nM to 5 $\mu$ M	0.001 to 100 $\mu$ M
No. of replicates	4	2
No. of times assay repeated	n.p.	3
No. of cells/well	100,000	20,000
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	ZR-75
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	3-4 days	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	10 $\mu$ M	10 nM to 10 $\mu$ M
No. of replicates	2	2
No. of times assay repeated	2	2
No. of cells/well	n.p.	n.p.
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	ZR-75
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	n.p.	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0.1 nM to 100 $\mu$ M	10 $\mu$ M
No. of replicates	n.p.	2
No. of times assay repeated	n.p.	2
No. of cells/well	2000	n.p.
<i>Agonism</i>		
Reference ligand	none	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	not done
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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## ER Cell Proliferation Assays

	Jones et al. (1998)	Korner et al. (1995)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	Overnight to attach + 48 hours	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	up to 0.1 $\mu$ M	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
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
	not done	not done
Reference ligand		
Final concentration of reference ligand		
Cell division/incubation		
Measured as (e.g., cell division)		

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## ER Cell Proliferation Assays

	Le Guevel and Pakdel (2001)	Makela et al. (1994)
<b>Characteristics of Cell Line</b>		
Cell line	Ishikawa	MCF-7
Cell source	human endometrial tumor	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	About 1 day	2 days
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	varies by substance (about $10^{-12}$ - $10^{-7}$ mol/L)	10 pM to 1 $\mu$ M
No. of replicates	n.p.	n.p.
No. of times assay repeated	6	8 - 12
No. of cells/well	20,000 cells/200 $\mu$ L	2000
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Ethinyl estradiol	17 $\beta$ -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
<i>Antagonism</i>		
	not done	
Reference ligand		17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	T47D
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	About 1 day	About 1 day
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1.0 pM to 1 $\mu$ M	1.0 fM to 1.0 $\mu$ M
No. of replicates	3	3
No. of times assay repeated	n.p.	n.p.
No. of cells/well	2000	10,000
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	n.p.	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	n.p.	0.5 - 20 $\mu$ M
No. of replicates	n.p.	4
No. of times assay repeated	n.p.	3
No. of cells/well	$5 \times 10^3$ cell/cm <sup>2</sup>	15,000
<i>Agonism</i>		
Reference ligand	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	17 $\beta$ -Estradiol
Final concentration of reference ligand		0.01 $\mu$ M
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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	24 hours	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	DMSO	n.p.
Range of test substance concentrations	0 -10 $\mu$ M	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
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	17 $\beta$ -Estradiol	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	24 hours	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	n.p.	n.p.
Range of test substance concentrations	1 nM to 500 $\mu$ M	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.
<i>Agonism</i>		
Reference ligand	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	24 hours after seeding + 72 hours	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.1 to 10 $\mu$ M	100 nM to 10 $\mu$ M
No. of replicates	3	3
No. of times assay repeated	2	n.p.
No. of cells/well	10,000	50,000
<i>Agonism</i>		
Reference ligand	none	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7 (subline BUS)	T47D
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	Overnight to 3 days	Overnight to 3 days
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1 nM to 5 $\mu$ M	1 nM to 5 $\mu$ M
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
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	ZR-75-1	MCF-7 (Bos)
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	Overnight to 3 days	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	Ethanol
Range of test substance concentrations	1 nM to 5 $\mu$ M	100 nM to 100 $\mu$ M
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
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	not done	17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	24 hours	24 hours
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol or DMSO	n.p.
Range of test substance concentrations	1 nM, 10 $\mu$ M	100 nM to 50 $\mu$ M
No. of replicates	2	2
No. of times assay repeated	At least 5	At least 5
No. of cells/well	20,000	20,000
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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)
<i>Antagonism</i>		
Reference ligand	not done	not done
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)
<b>Characteristics of Cell Line</b>		
Cell line	T47D	MCF-7
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	48 hours	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.1 nM to 25 $\mu$ M	1, 10, 25 $\mu$ M
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.
<i>Agonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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
<i>Antagonism</i>		
Reference ligand	17 $\beta$ -Estradiol	17 $\beta$ -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)
<b>Characteristics of Cell Line</b>		
Cell line	MCF-7 (E3 clone)	MCF-7 (E3 clone)
Cell source	human breast cancer	human breast cancer
<b>Preparation of Cells for Assay</b>		
Plating time prior to treatment with test substance	5 days	n.p.
<b>Cell Proliferation Assay</b>		
Test substance solvent	Ethanol	n.p.
Range of test substance concentrations	0.001, 0.1, 1, and 10 $\mu$ M	1 pM to 1 $\mu$ M
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
<i>Agonism</i>		
Reference ligand	17 -Estradiol	17 -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
<i>Antagonism</i>		
Reference ligand	17 -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