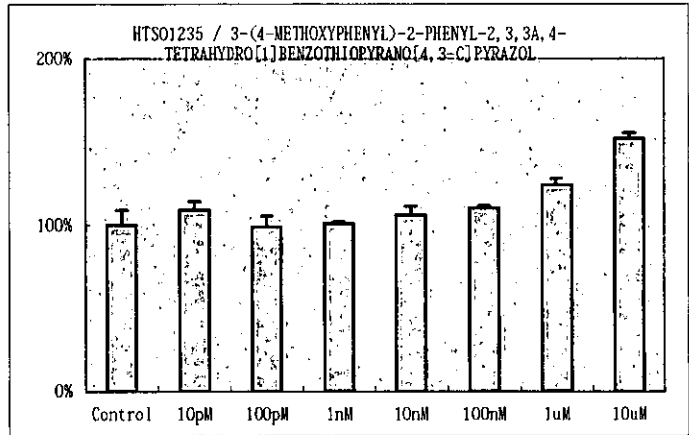


ER α /HeLa Antagonist

HTS01235

3-(4-METHOXYPHENYL)-2-PHENYL-2,3,3A,4-TETRAHYDRO[1]BENZOTHIOPYRANO[4,3-C]PYRAZOL

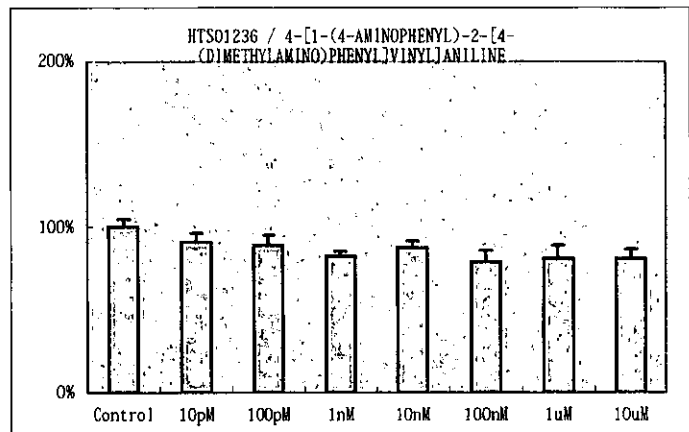
IC50 (pM): -



HTS01236

4-[1-(4-AMINOPHENYL)-2-[4-(DIMETHYLAMINO)PHENYL]VINYL]ANILINE

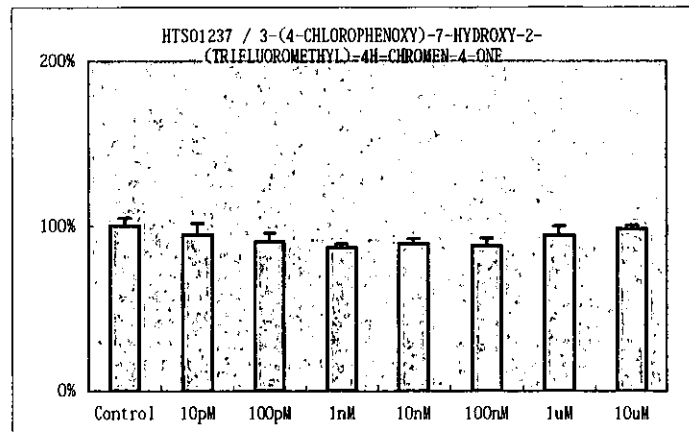
IC50 (pM): -



HTS01237

3-(4-CHLOROPHENOXY)-7-HYDROXY-2-(TRIFLUOROMETHYL)-4H-CHROMEN-4-ONE

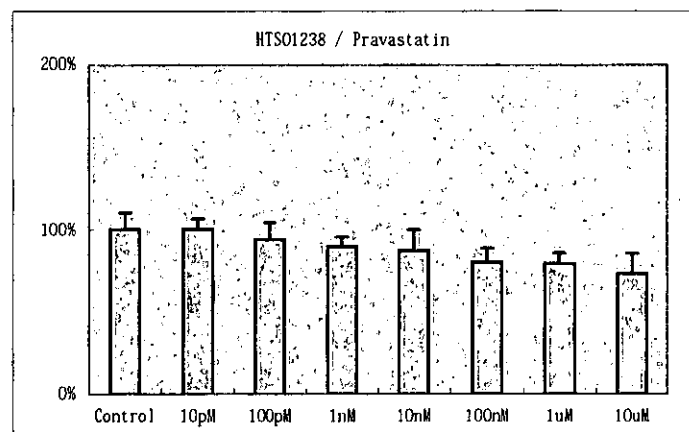
IC50 (pM): -



HTS01238

Pravastatin

IC50 (pM): -

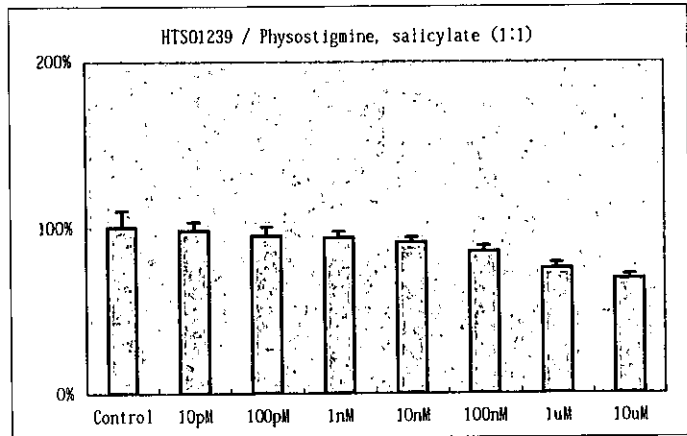


ER α /HeLa Antagonist

HTS01239

Physostigmine, salicylate (1:1)

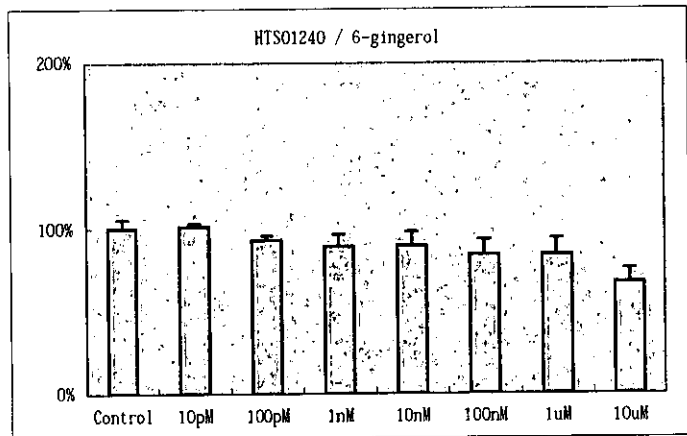
IC50 (pM): -



HTS01240

6-gingerol

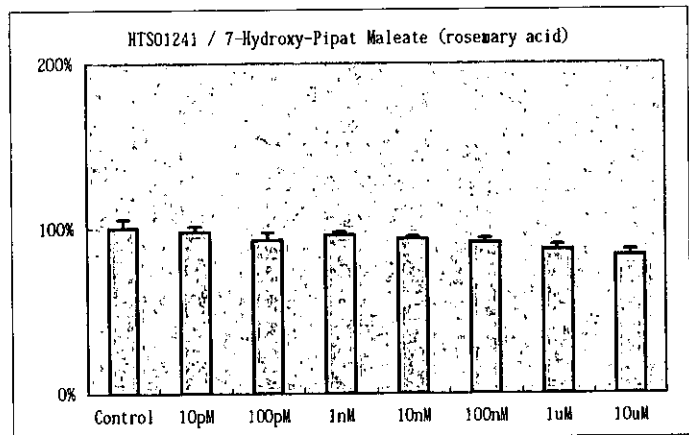
IC50 (pM): -



HTS01241

7-Hydroxy-Pipat Maleate (rosemary acid)

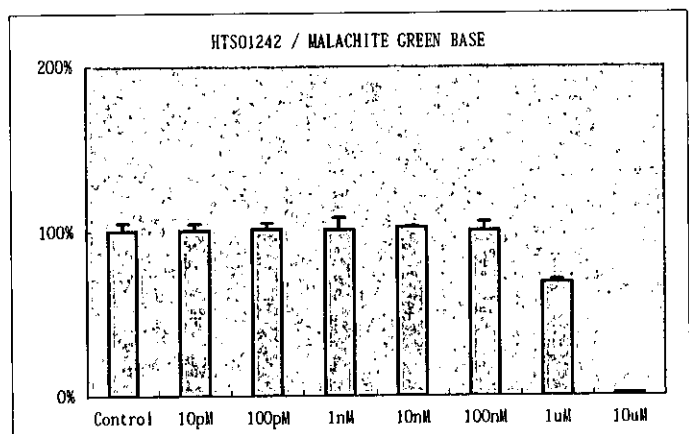
IC50 (pM): -



HTS01242

MALACHITE GREEN BASE

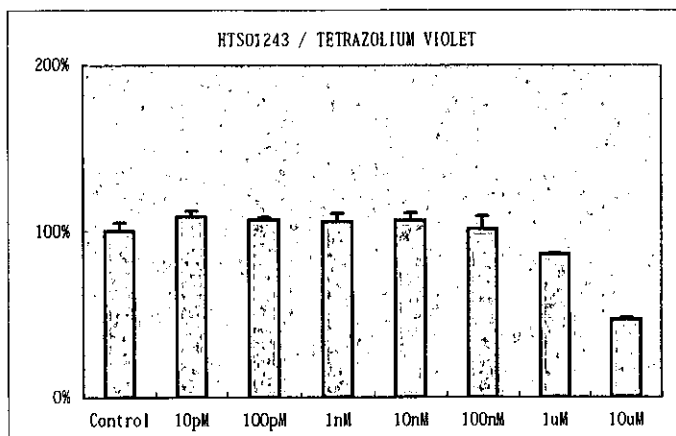
IC50 (pM): 1.07E+06



ER α /HeLa Antagonist

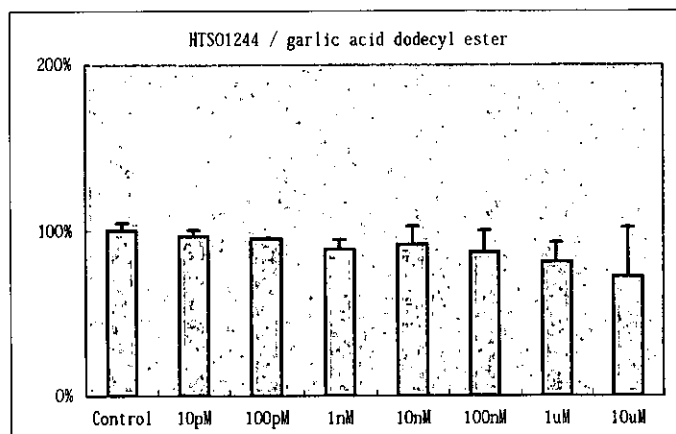
HTS01243
TETRAZOLIUM VIOLET

IC50 (pM): -



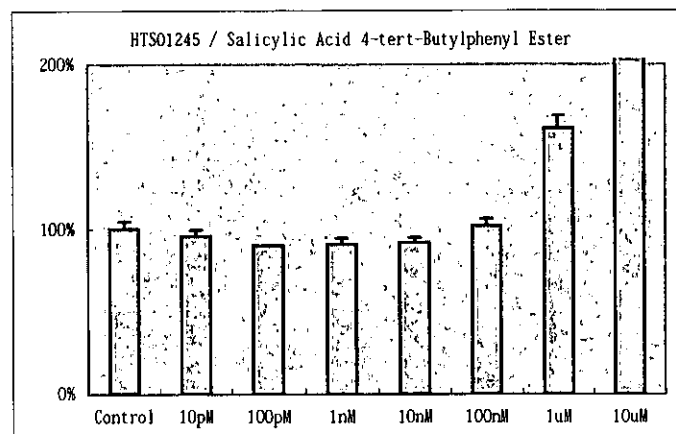
HTS01244
garlic acid dodecyl ester

IC50 (pM): -



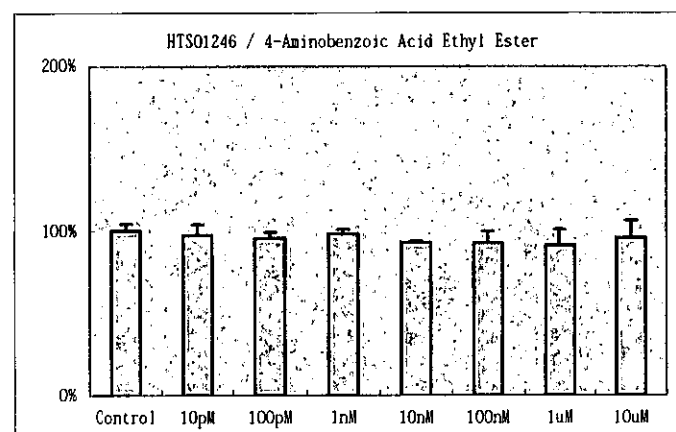
HTS01245
Salicylic Acid 4-tert-Butylphenyl Ester

IC50 (pM): -



HTS01246
4-Aminobenzoic Acid Ethyl Ester

IC50 (pM): -

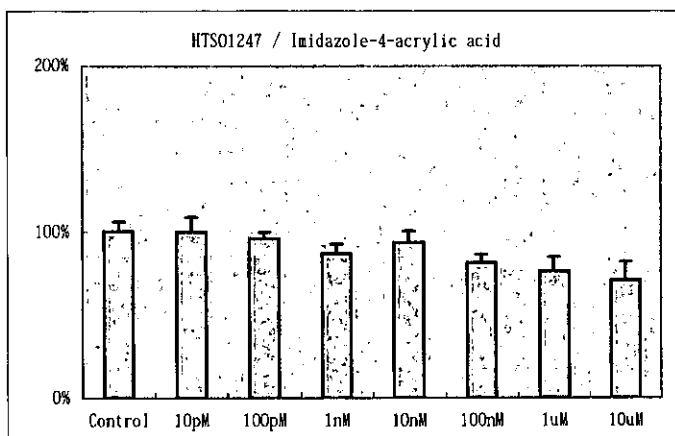


ER α /HeLa Antagonist

HTS01247

Imidazole-4-acrylic acid

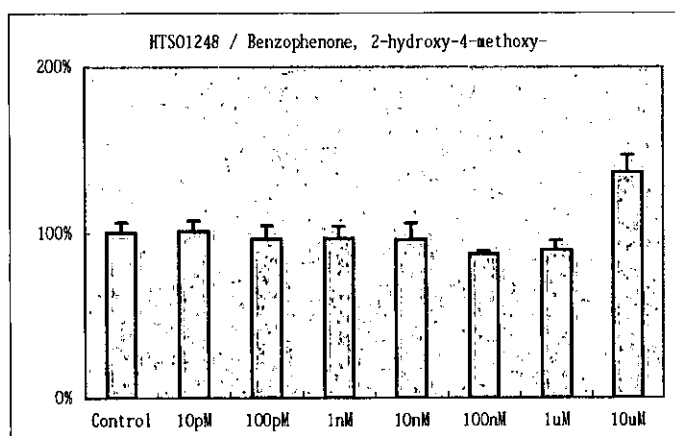
IC50 (pM): -



HTS01248

Benzophenone, 2-hydroxy-4-methoxy-

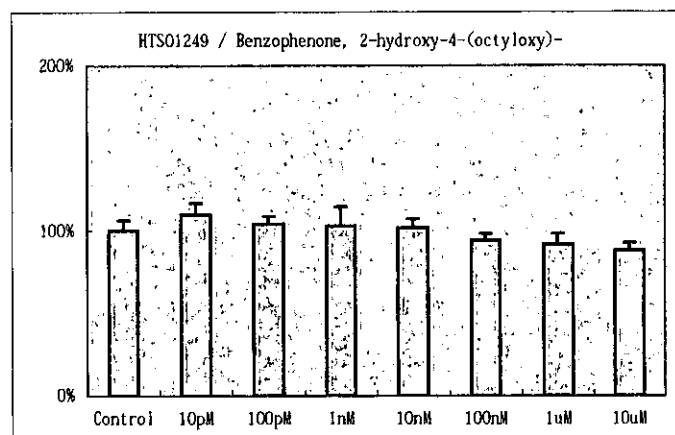
IC50 (pM): -



HTS01249

Benzophenone, 2-hydroxy-4-(octyloxy)-

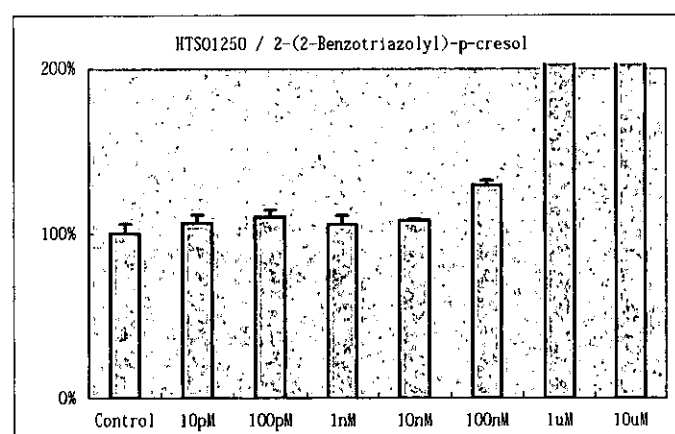
IC50 (pM): -



HTS01250

2-(2-Benzotriazolyl)-p-cresol

IC50 (pM): -

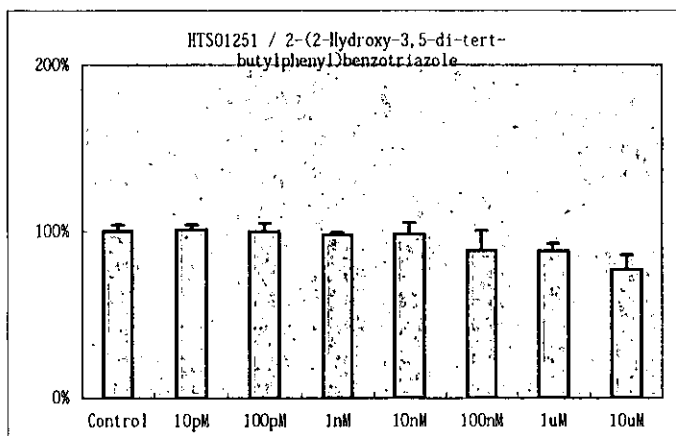


ER α /HeLa Antagonist

HTS01251

2-(2-Hydroxy-3,5-di-tert-butylphenyl)benzotriazole

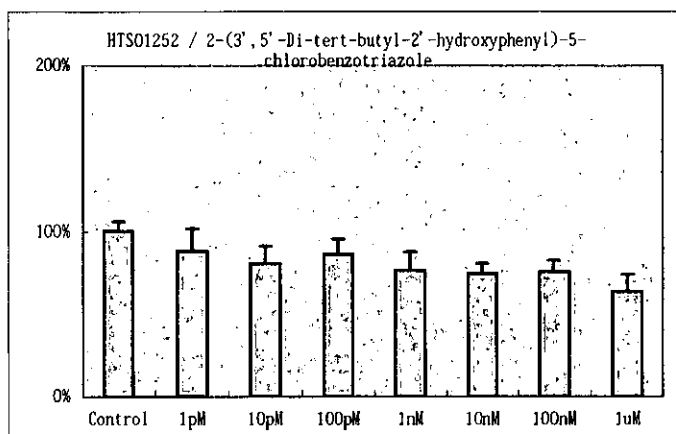
IC50 (pM): -



HTS01252

2-(3',5'-Di-tert-butyl-2'-hydroxyphenyl)-5-chlorobenzotriazole

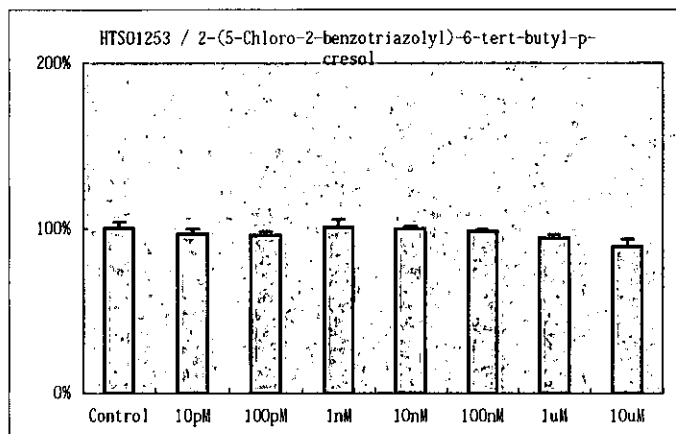
IC50 (pM): -



HTS01253

2-(5-Chloro-2-benzotriazolyl)-6-tert-butyl-p-cresol

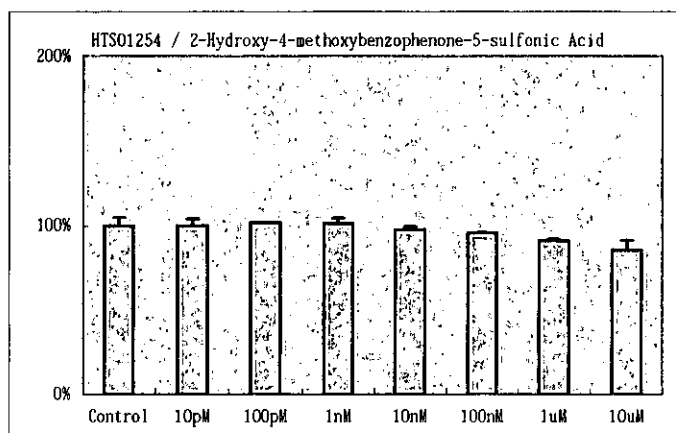
IC50 (pM): -



HTS01254

2-Hydroxy-4-methoxybenzophenone-5-sulfonic Acid

IC50 (pM): -

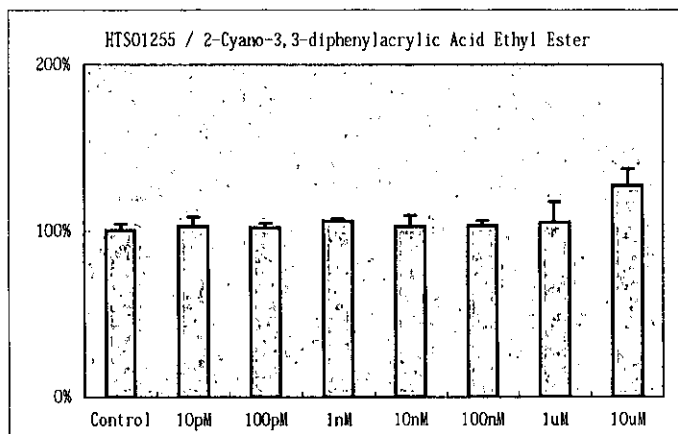


ER α /HeLa Antagonist

HTS01255

2-Cyano-3,3-diphenylacrylic Acid Ethyl Ester

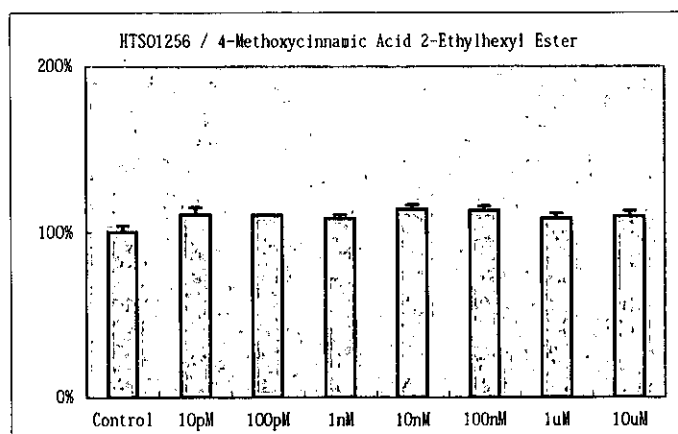
IC50 (pM): -



HTS01256

4-Methoxycinnamic Acid 2-Ethylhexyl Ester

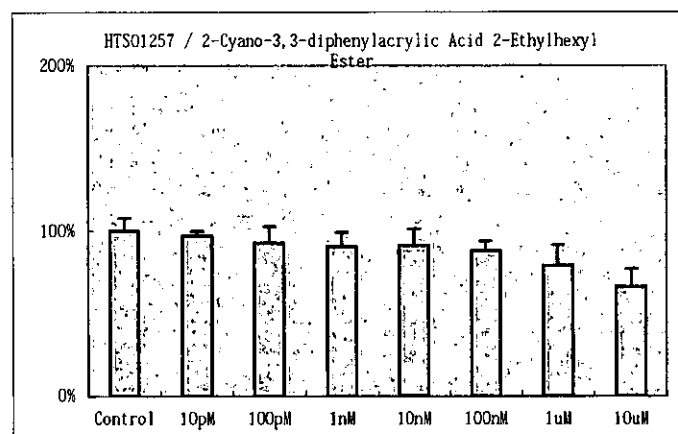
IC50 (pM): -



HTS01257

2-Cyano-3,3-diphenylacrylic Acid 2-Ethylhexyl Ester

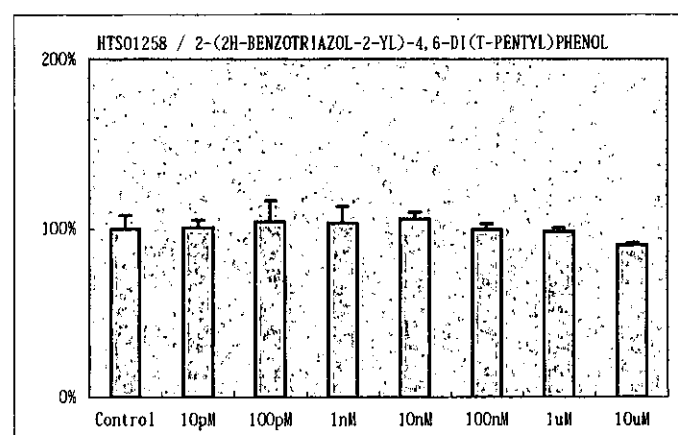
IC50 (pM): -



HTS01258

2-(2H-BENZOTRIAZOL-2-YL)-4,6-DI(T-PENTYL)PHENOL

IC50 (pM): -

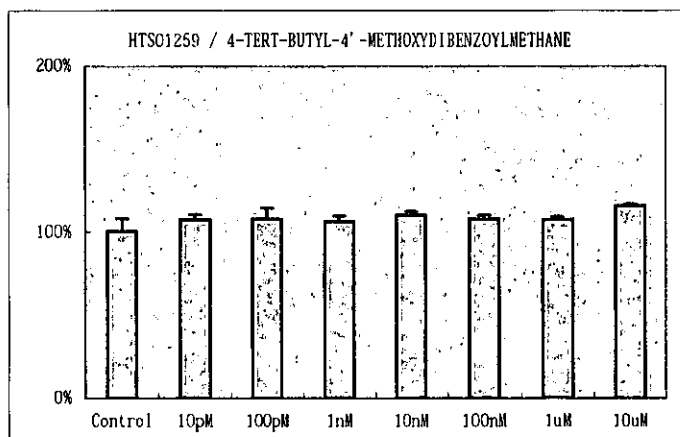


ER α /HeLa Antagonist

HTS01259

4-TERT-BUTYL-4'-METHOXYDIBENZOYLMETHANE

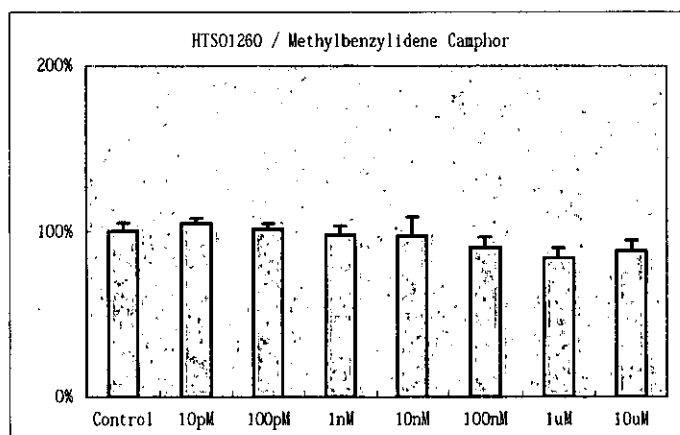
IC50 (pM): -



HTS01260

Methylbenzylidene Camphor

IC50 (pM): -



ID	CAS No.	Name	M.W.	PC10	PC50	IC50
EA120	-	4-(4-CHLOROPHENYL)-5-(4-METHYLPHENYL)-1,3-THIAZOL-2-AMINE HYDROBROMIDE	381.7	6.42E+03	-	-
EA121	-	N1-(4-PYRIDYL)-5-NITRO-2-(PHENYLTHIO)BENZAMIDE	351.4	-	-	-
EA133	-	3-(4-CHLOROPHENYL)-4-((4-CHLOROPHENYL)THIO)-1H-PYRAZOLE	321.2	-	-	-
EA148	-	5-[2,5-DI(2,2,2-TRIFLUOROETHOXY)PHENYL]-1H-PYRAZOLE	340.2	-	-	-
EA151	-	N3-(4-BROMOPHENYL)-4,5-DIOXO-2-PHENYL-4,5-DIHYDRO-1H-PYRROLE-3-CARBOXAMIDE	371.2	1.00E+06	-	-
EA160	-	3-(4-HYDROXY-3-METHOXYBENZYLIDENE)-2-PHENYLCHROMAN-4-ONE	358.4	4.07E+04	3.78E+05	-
EA161	-	N1-[3-(4-HYDROXYANILINO)-2-QUINOXALINYL]-4-METHYL-1-BENZENESULFONAMIDE	406.5	-	-	-
EA164	-	6-[(ETHYLTHIO)METHYL]-2-[4-(TRIFLUOROMETHYL)PHENYL]PYRIMIDIN-4-OL	314.3	-	-	-
EA165	-	DIETHYL 2-[(1H-1,2,3-BENZOTRIAZOL-6-YLAMINO)METHYLIDENE]MALONATE	304.3	-	-	-
EA166	-	5-(3,4-DIMETHOXYPHENYL)-1,3-DIPHENYL-4,5-DIHYDRO-1H-PYRAZOLE	358.4	-	-	-
EA205	-	5,7-DIHYDROXY-2,3-DIPHENYL-4H-CHROMEN-4-ONE	330.3	2.64E+03	2.44E+04	-
EA207	-	4-[2-(4-CHLOROPHENYL)-2,3-DIHYDRO-1,5-BENZOTHAZEPIN-4-YL]PHENOL	365.9	1.95E+05	-	-
EA209	-	2-[2-(4-HYDROXYPHENYL)-2,3-DIHYDRO-1,5-BENZOTHAZEPIN-4-YL]PHENOL	347.4	1.24E+05	5.51E+05	-
EA210	-	4-[2-[4-(2,4-DIMETHYLPHENYL)-5-MERCAPTO-4H-1,2,4-TRIAZOL-3-YL]ETHYL]PHENOL	325.4	-	-	-
EA214	-	7-HYDROXY-3-(4-METHOXYPHENYL)-2-PHENYL-4H-CHROMEN-4-ONE	344.4	1.01E+05	3.71E+05	-
EA222	-	5-(1-NAPHTHYL)-1,3-DIPHENYL-4,5-DIHYDRO-1H-PYRAZOLE	348.4	-	-	-
EA224	-	5-(4-ETHOXY-3-METHOXYPHENYL)-1,3-DIPHENYL-4,5-DIHYDRO-1H-PYRAZOLE	372.5	-	-	-
EA226	-	N1-(2-METHYL-3-NITROPHENYL)-N1-[(2-METHYL-3-NITROPHENYL)IMINO]METHYL]BENZAMIDE	418.4	-	-	-
EA228	-	METHYL 4-[[7-HYDROXY-4-OXO-2-(TRIFLUOROMETHYL)-4H-3-CHROMENYL]OXY]BENZOATE	380.3	5.76E+04	3.13E+05	-
EA240	-	N1-(2-[5-[4-(TERT-BUTYL)PHENYL]-1,3,4-OXADIAZOL-2-YL]PHENYL)-3-METHYLBENZAMIDE	414.4	9.72E+06	-	-
EA258	-	ZP	360.9	-	-	-
EA260	-	3-(2-CHLOROPHENYL)-5-(4-METHYLPHENYL)-1-PHENYL-4,5-DIHYDRO-1H-PYRAZOLE	346.9	-	-	-
EA263	-	2-[3-(4-METHOXYPHENYL)-3,3A,4,5-TETRAHYDRO-2H-BENZO[G]INDAZOL-2-YL]ETHYL CYCLOPR	390.5	1.18E+06	-	-
EA318	-	3-(4-METHOXYPHENYL)-2-PHENYL-2,3,3A,4-TETRAHYDRO[1]BENZOTHIOPYRANO[4,3-C]PYRAZOL	372.5	2.23E+06	-	-
EA322	-	4-[1-(4-AMINOPHENYL)-2-[4-(DIMETHYLAMINO)PHENYL]VINYL]ANILINE	329.4	-	-	-
EA326	-	3-(4-CHLOROPHENOXY)-7-HYDROXY-2-(TRIFLUOROMETHYL)-4H-CHROMEN-4-ONE	356.7	-	5.56E+05	-

ER529	1093-37-0	Pravastatin	446.5	-	-	-
ER530	57-64-7	Physostigmine, salicylate (1:1)	413.5	-	-	-
ER531	64-86-8	Colchicine	399.4	8.55E+04	-	-
ER532	500-38-9	NORDIHYDROGUAIARETIC ACID	302.4	-	-	-
ER533	50-55-5	Reserpine	608.7	-	-	-
ER534	596-27-0	o-Cresolphthalein	346.4	-	-	-
ER535	74-31-7	p-Phenylenediamine, N,N-diphenyl-	260.3	2.30E+06	-	-
ER536	99-65-0	1,3-Dinitrobenzene	168.1	-	-	-
ER539	303-45-7	gossypol	578.6	-	-	-
ER541	3513-14-6	6-gingerol	294.4	-	-	-
ER542	0283-92-5	7-Hydroxy-Pipat Maleate (rosemary acid)	360.3	-	-	-
ER543	77-09-8	PHENOLPHTHALEIN	318.3	-	-	-
ER544	510-13-4	MALACHITE GREEN BASE	346.5	7.59E+04	-	1.07E+06
ER545	3248-91-7	NEW FUCHSIN	365.9	-	-	-
ER546	1719-71-7	TETRAZOLIUM VIOLET	384.9	-	-	-
ER549	6948-88-5	ALPHA-NAPHTHOLBENZEIN	377.4	4.89E+01	2.43E+05	-
ER550	1166-52-5	garlic acid dodecyl ester	338.4	-	-	-
ER551	2716-10-1	ALPHA,ALPHA'-BIS(4-AMINOPHENYL)-1,4-DIISOPROPYLBENZENE	344.5	-	-	-
ER600	87-18-3	Salicylic Acid 4-tert-Butylphenyl Ester	270.3	-	8.47E+05	-
ER601	94-9-7	4-Aminobenzoic Acid Ethyl Ester	165.2	2.09E+03	-	-
ER603	104-98-3	Imidazole-4-acrylic acid	138.1	-	-	-
ER604	118-55-8	Salicylic acid, phenyl ester	214.2	-	-	-
ER606	118-60-5	Salicylic acid, 2-ethylhexyl ester	250.3	-	-	-
ER607	131-54-4	2,2'-Dihydroxy-4,4'-dimethoxybenzophenone	274.3	2.23E+05	7.62E+06	-
ER608	131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	246.2	1.06E+05	3.28E+05	-
ER609	131-56-6	2,4-Dihydroxybenzophenone	214.2	1.61E+05	2.41E+06	-

ER610	131-57-7	Benzophenone, 2-hydroxy-4-methoxy-	228.2	3.55E+05	5.82E+06	-
ER612	1843-05-6	Benzophenone, 2-hydroxy-4-(octyloxy)-	326.4	-	-	-
ER613	2440-22-4	2-(2-Benzotriazolyl)-p-cresol	225.3	1.01E+04	1.05E+06	-
ER617	3846-71-7	2-(2-Hydroxy-3,5-di-tert-butylphenyl)benzotriazole	323.4	-	-	-
ER618	3864-99-1	2-(3',5'-Di-tert-butyl-2'-hydroxyphenyl)-5-chlorobenzotriazole	357.9	-	-	-
ER619	3896-11-5	2-(5-Chloro-2-benzotriazolyl)-6-tert-butyl-p-cresol	315.8	-	-	-
ER620	4065-45-6	2-Hydroxy-4-methoxybenzophenone-5-sulfonic Acid	308.3	-	-	-
ER622	5232-99-5	2-Cyano-3,3-diphenylacrylic Acid Ethyl Ester	277.3	1.22E+06	6.43E+06	-
ER624	5466-77-3	4-Methoxycinnamic Acid 2-Ethylhexyl Ester	290.4	-	-	-
ER625	6197-30-4	2-Cyano-3,3-diphenylacrylic Acid 2-Ethylhexyl Ester	361.5	9.69E+06	-	-
ER627	1245-02-3	4-Dimethylaminobenzoic Acid 2-Ethylhexyl Ester	277.4	-	-	-
ER628	5973-55-1	2-(2H-BENZOTRIAZOL-2-YL)-4,6-DI(T-PENTYL)PHENOL	351.5	1.04E+06	-	-
ER630	0356-09-1	4-TERT-BUTYL-4'-METHOXYDIBENZOYLMETHANE	310.4	-	-	-
ER636	6861-47-9	Methylbenzylidene Camphor	254.4	4.44E+06	-	-

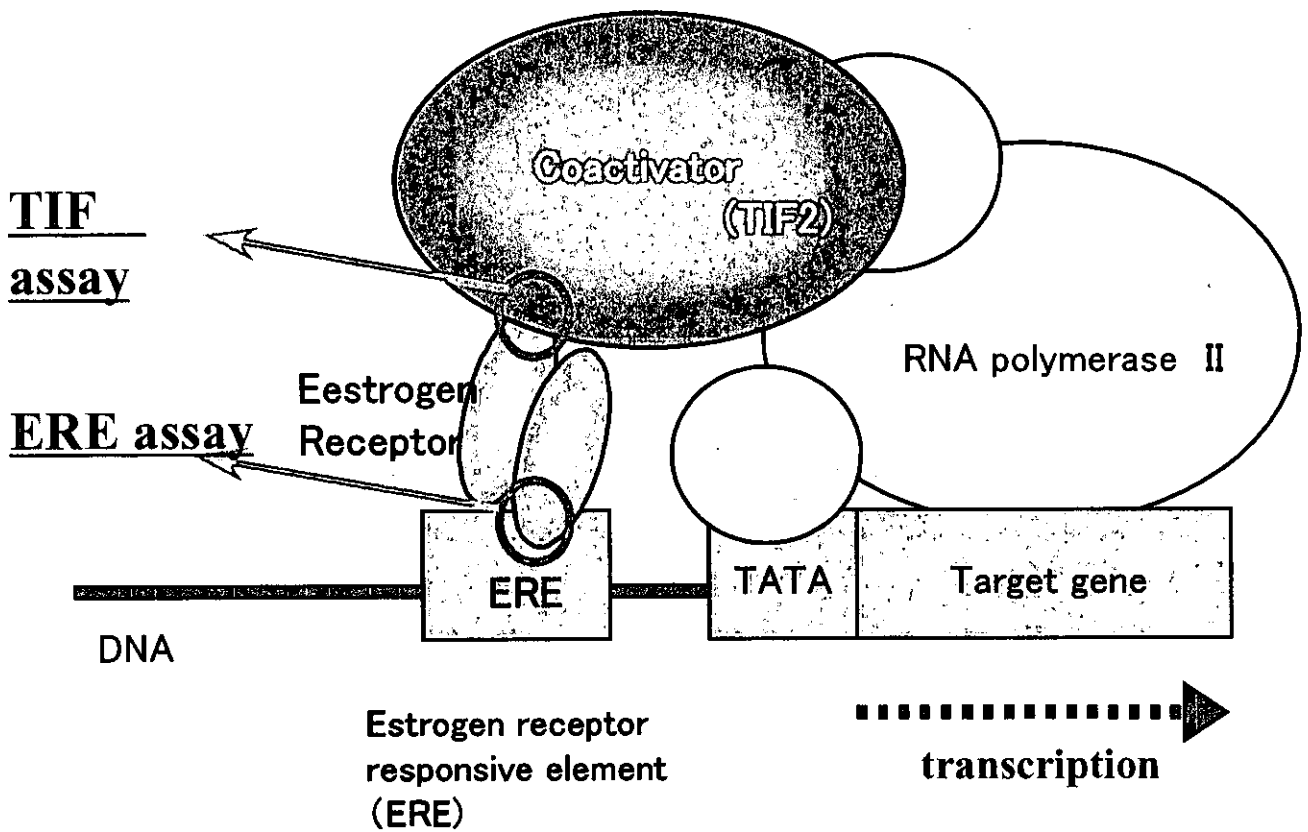


図1-1

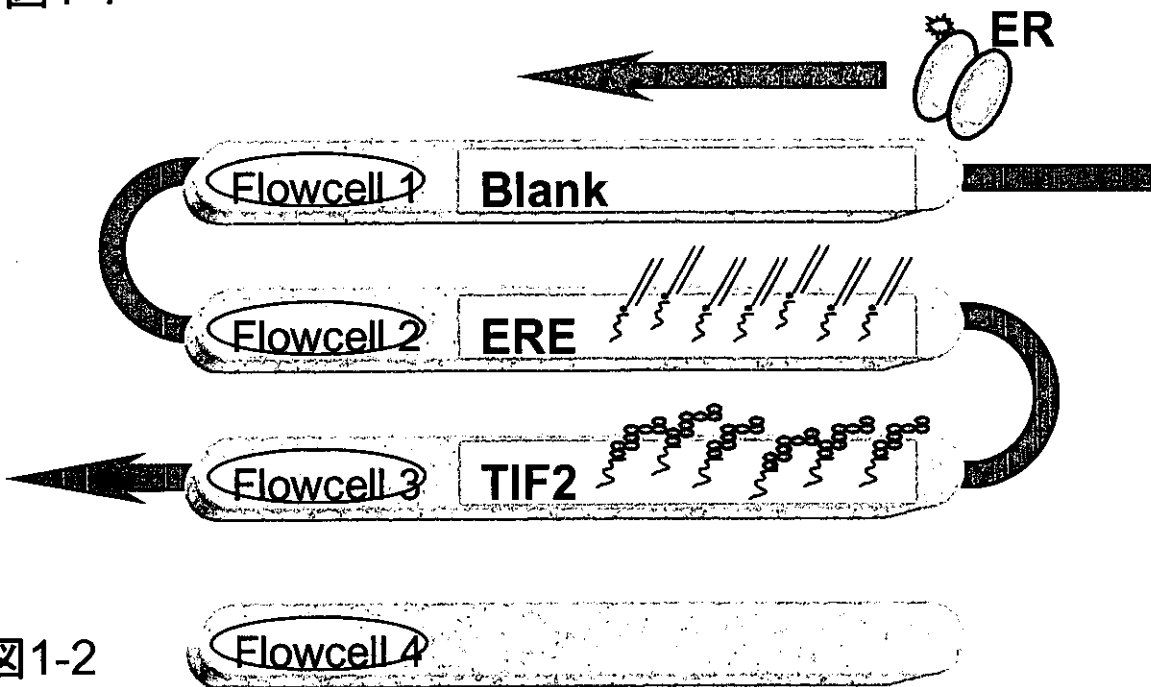
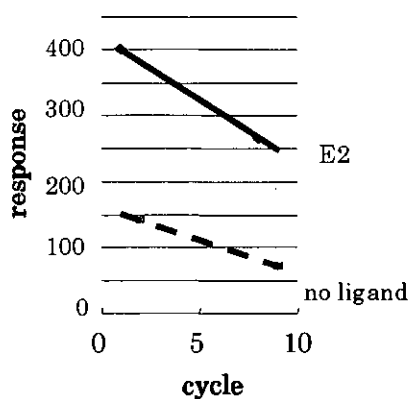
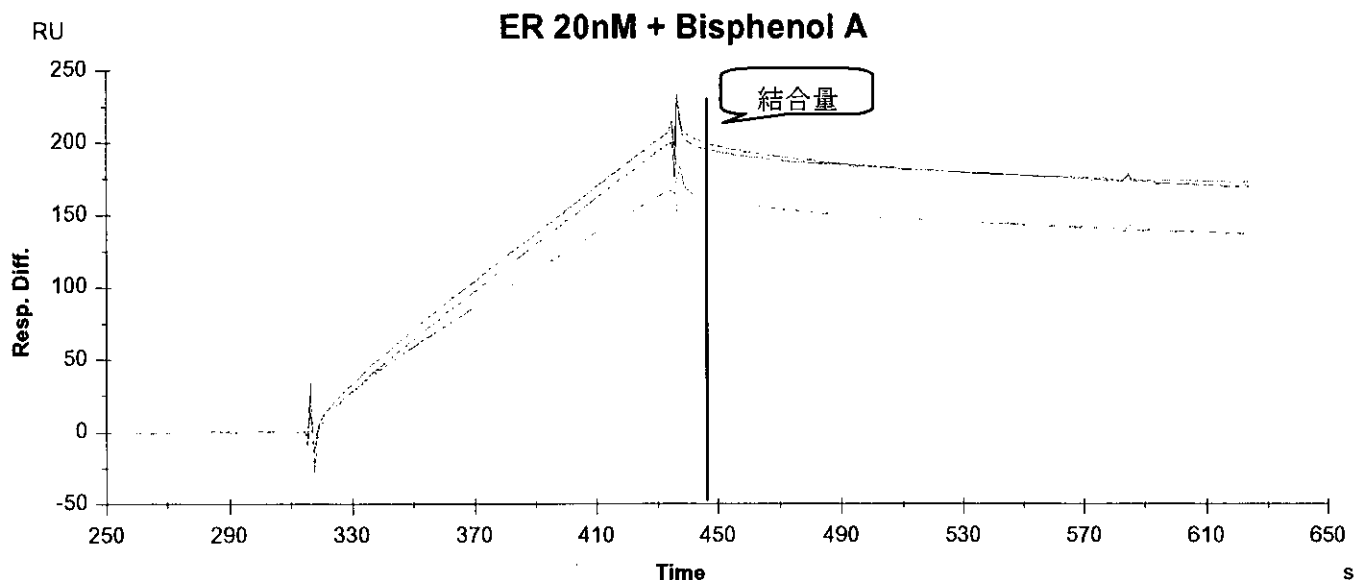


図1-2

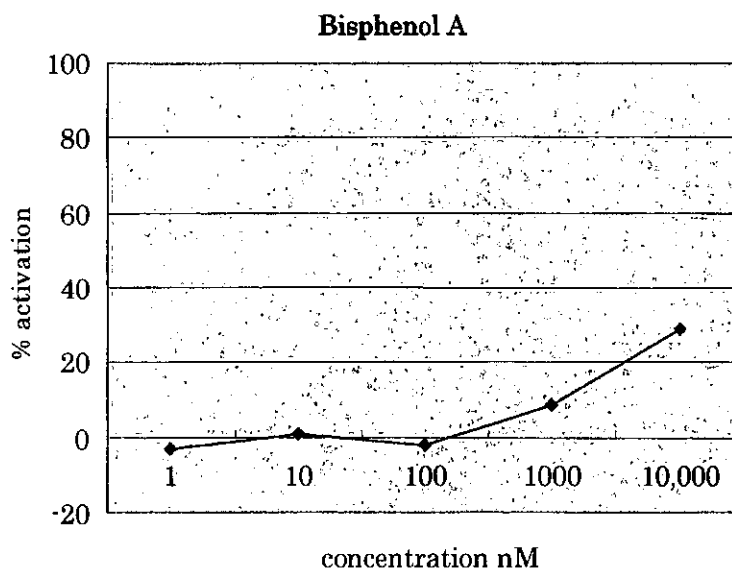
図1 表面プラズモン共鳴スクリーニング法の概要

図1-1 エストロゲンレセプターの作用機構と2種類のアッセイ

図1-2 表面プラズモン共鳴センサー上でのアッセイの構築



cycle	bisphenol A		BIA9				
	conc nM	RelRes	E2cal	nolig cal	drug activation	E2 activation	%activation
2	0	141.4	380.75	141.40	0.00	239.36	0.00
9	0	70.8	246.05	70.80	0.00	175.26	0.00
7	1	84.8	284.54	90.97	-6.17	193.57	-3.19
6	10	92.1	284.54	90.97	1.13	193.57	0.58
5	100	97.2	303.78	101.05	-3.85	202.73	-1.90
4	1000	129	323.03	111.14	17.86	211.89	8.43
3	10,000	185.5	342.27	121.23	64.27	221.04	29.08
1	100	400	400.00	151.48	248.52	248.51	100.00
8	100	265.3	265.30	80.88	184.42	184.41	100.00



$$\% \text{ activation} = \frac{(\text{化学物質存在下での結合量} - \text{ネガティブコントロールでの結合量})}{(100 \text{ nM E2存在下での結合量} - \text{ネガティブコントロールでの結合量})} \times 100$$

図2 ERアッセイのデータ解析法

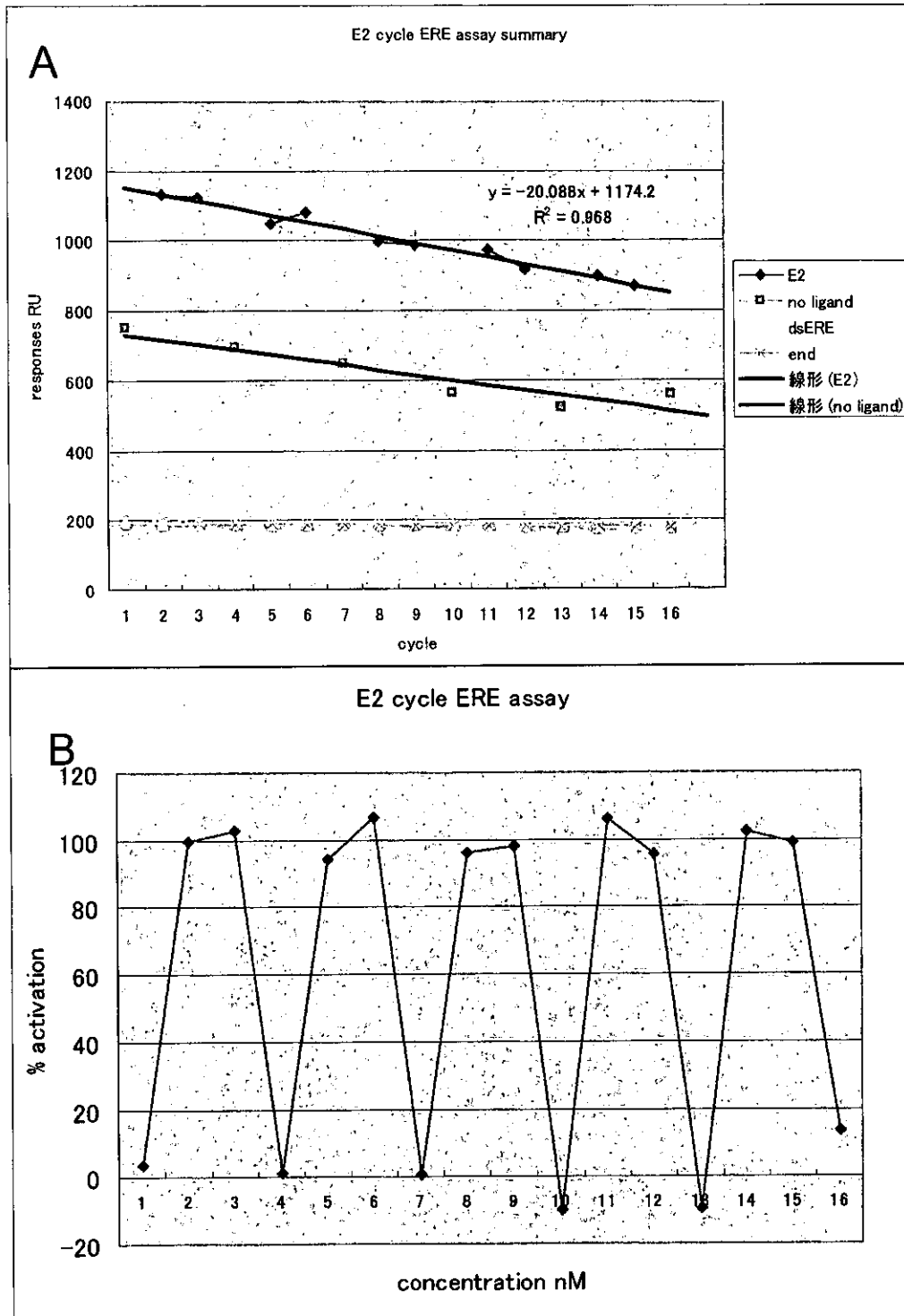


図3 アッセイの再現性 ERE アッセイ

アッセイの再現性を確認するためにポジティブコントロールとネガティブコントロールを繰り返し16サイクル測定した。

ポジティブコントロール: サイクル2,3,5,6,8,9,11,12,14,15

ネガティブコントロール: サイクル1,4,7,10,13,16

A: 各サイクルの結合量、ERE ハイブリダイズ量、アッセイ終了時のベースラインの値を示す。ハイブリダイズしたEREが再生後ほとんどすべてセンサーチップからはずれている。

B: ER活性の補正後、各サイクルの相対活性化度をプロットした。

ポジティブコントロール 平均値 100.0% 標準偏差 4.29%

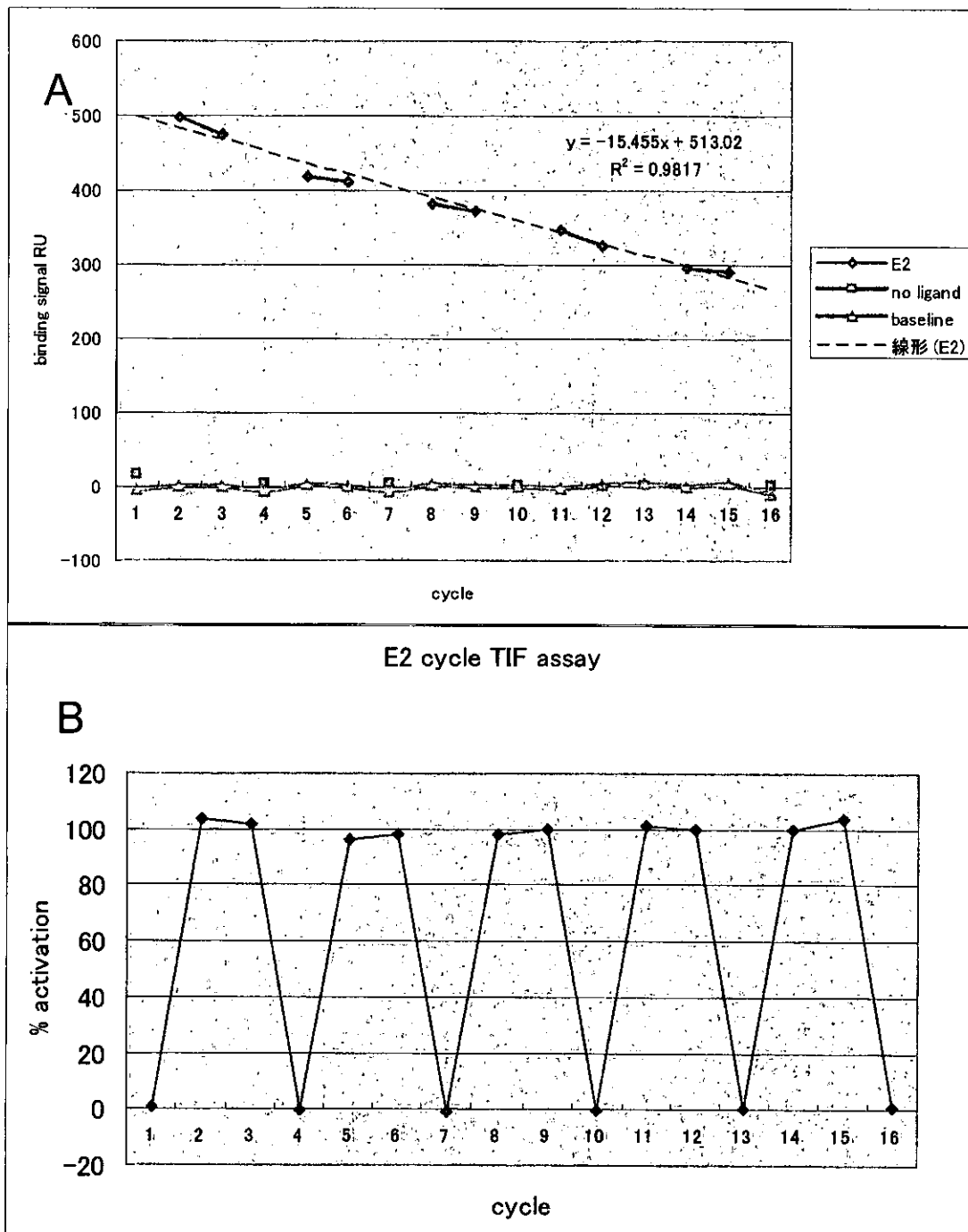


図4 アッセイの再現性 TIFアッセイ

アッセイの再現性を確認するためにポジティブコントロールとネガティブコントロールを繰り返し16サイクル測定した。

ポジティブコントロール: サイクル2,3,5,6,8,9,11,12,14,15

ネガティブコントロール: サイクル1,4,7,10,13,16

A: 各サイクルの結合量、アッセイ終了時のベースラインの値を示す。

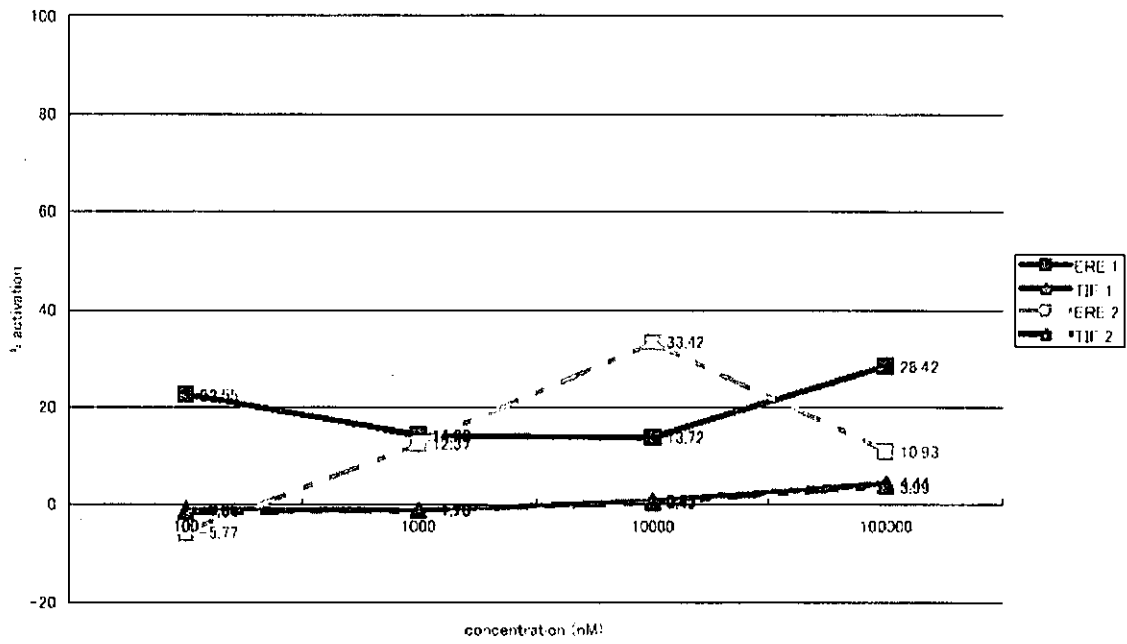
B: ER活性の補正後、各サイクルの相対活性化度をプロットした。

ポジティブコントロール 平均値 100.4% 標準偏差 2.36%

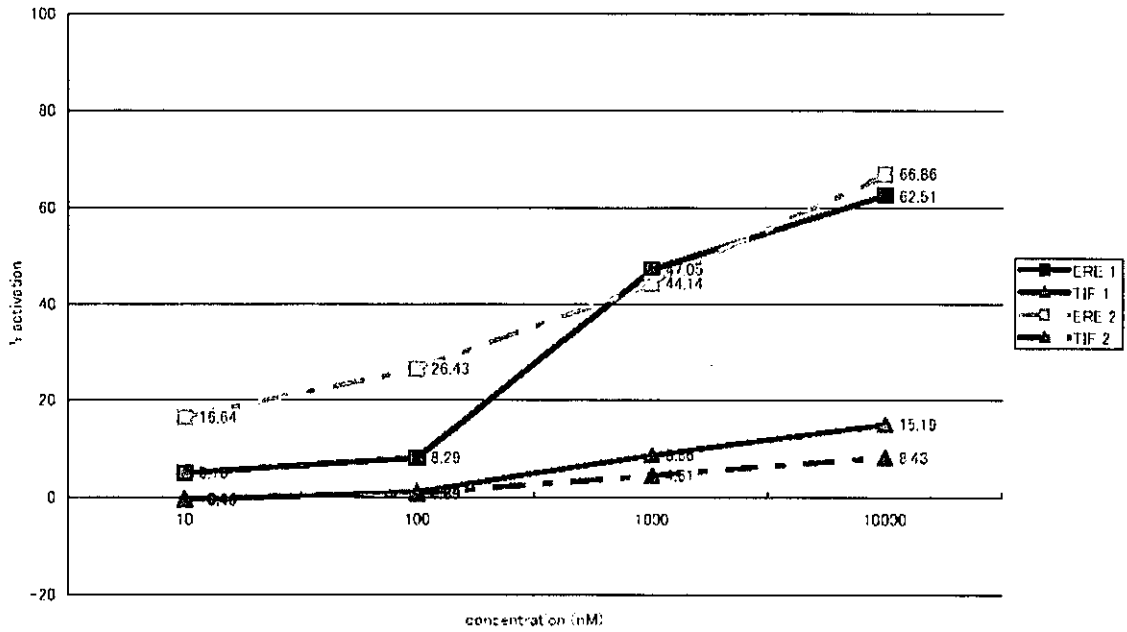
図5 平成14年度の90化合物のスクリーニング結果

EREアッセイとTIFアッセイを各2回行った結果を示す

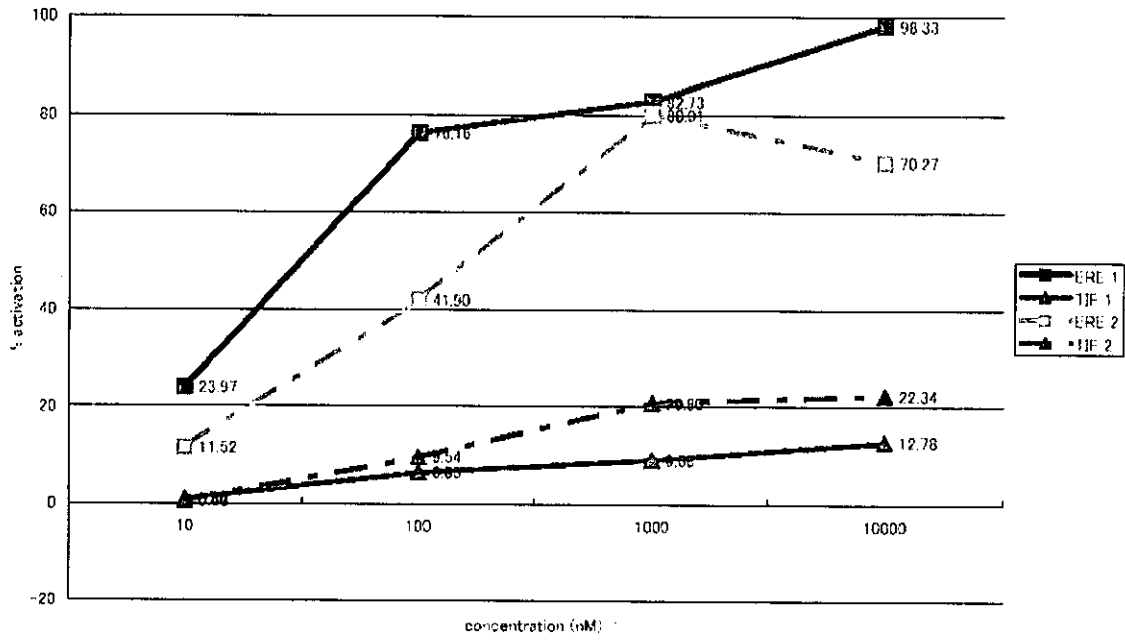
ER069



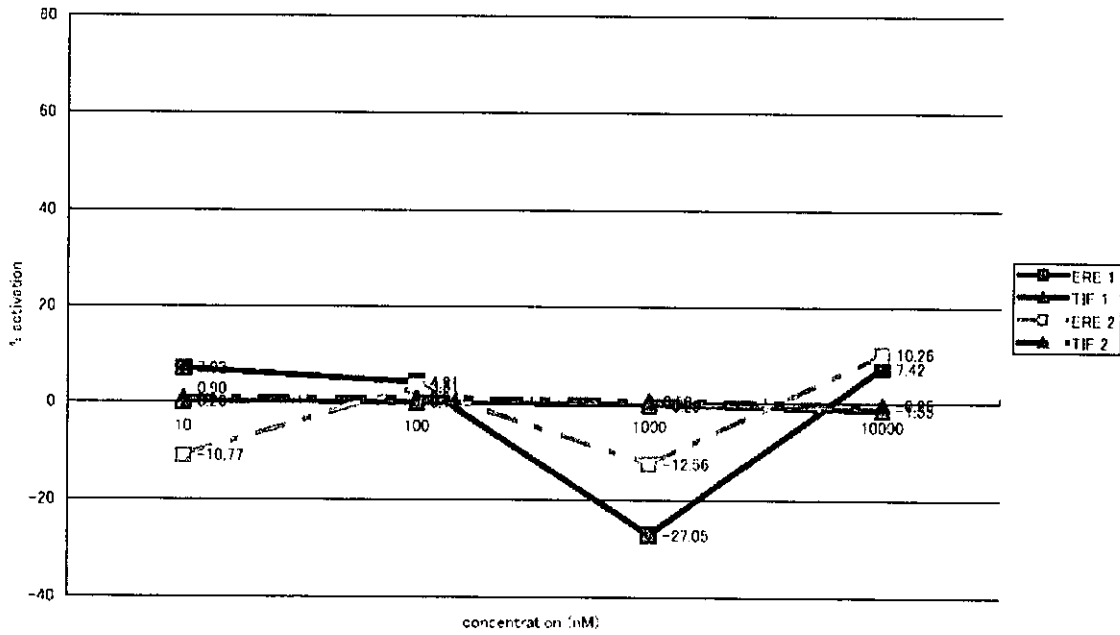
ER205

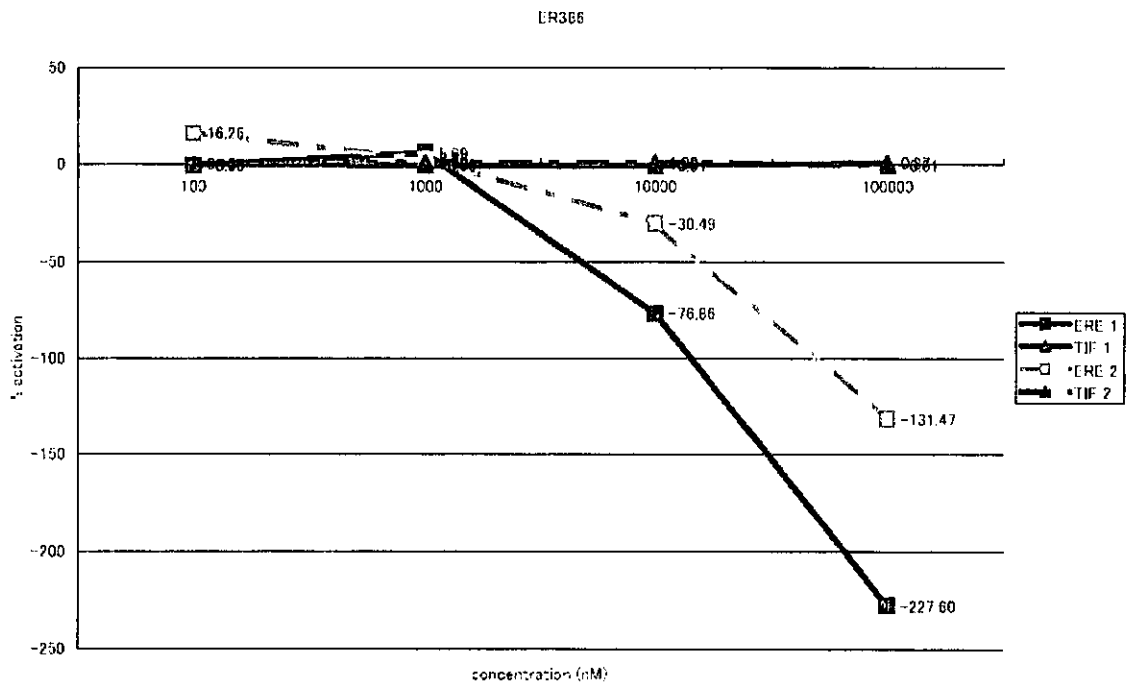
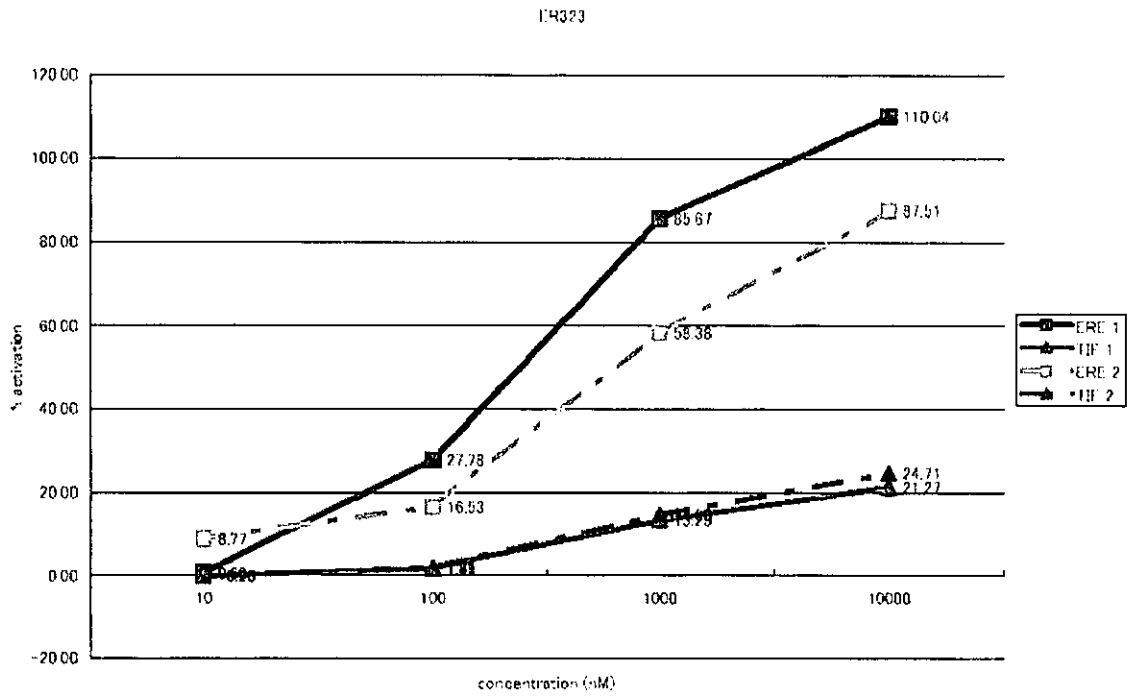


ER258

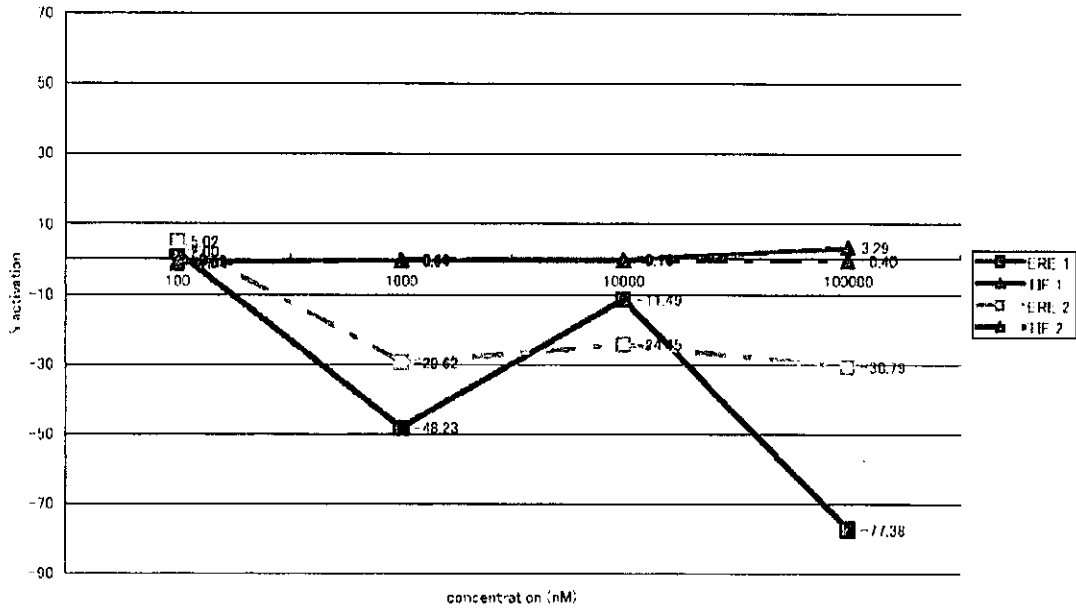


ER292





ER407



ER411

