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LAMPIRAN

Lampiran. Perhitungan jumlah sel untuk kultur pada 96-*well plate*

Jumlah sel dalam kamar hitung = $\frac{34+30+40+30}{4} = 33\ x\ 10^4 x2 = 66\ x\ 10^4$/mL

*2 = faktor dilusi karena penggunaan Trypan Blue sebanyak 10 μL

Volume total sel yang dibutuhkan pada 96-*well plate* (dibulatkan menjadi 100 sumuran) = 5.000 x 100 = 5 x 10^5

Volume medium kultur yang dibutuhkan pada 96-*well plate*= 100 x 100 = 10^4 = 10 mL

Volume sel yang diambil = $\frac{5\ x\ 10^5}{6{,}6\ x10^5} = 0{,}78\ mL = 780\ \mu L$ ad. Medium kultur 10 mL.

Lampiran. Uji Sitotoksisitas Ekstrak Etanol Daun Prasman

1. Pembuatan Larutan Baku Ekstrak Etanol Daun Prasman

   Ekstrak etanol daun Prasman ditimbang seberat 100 mg dan kemudian dilarutkan dengan DMSO 200 μL dan medium kultur 800 μL. Proses kelarutan dan homogenisasi dibantu dengan vortex selama 5 – 10 menit. Dosis yang diperoleh adalah 10 mg/ 1 mL = 10.000 μg/mL = 100.000 ppm (DMS0 20%)

   Larutan baku kemudian diencerkan lagi untuk mendapatkan konsentrasi 10.000 μg/mL dengan konsentrasi DMSO 2%

   V1 = 10.000/100.000 x 1 mL = 0,1 mL = 100 μL ad medium kultur 1000 μL.

2. Pembuatan Larutan Standar

   Pembuatan larutan standar setiap konsentrasi mengikuti persamaan

   C1 x V1 = C2 x V2

   Ket : C1 = konsentrasi awal

   V1 = volume awal

   C2 = konsentrasi hasil pengenceran

   V2 = volume larutan hasil pengenceran

Volume larutan hasil pengenceran yang ditetapkan untuk pengujian adalah 400 μL.

Konsentrasi (C2)	Volume ekstrak yang ditambahkan (V1)	Volume MK (ad 400 μL)	Konsentrasi DMSO
50 μg/mL	2 μL	398 μL	0,01 %
100 μg/mL	4 μL	396 μL	0,02 %
200 μg/mL	8 μL	392 μL	0,04 %
300 μg/mL	12 μL	388 μL	0,06 %
400 μg/mL	16 μL	384 μL	0,08 %
500 μg/mL	20 μL	380 μL	0,1 %
600 μg/mL	24 μL	376 μL	0,12 %
800 μg/mL	32 μL	368 μL	0,16 %

3. Pembuatan Larutan Baku Doxorubicin

Larutan Baku yang dijual dalam kemasan memiliki konsentrasi 2000 μg/mL. Konsentrasi larutan baku yang akan digunakan dalam penelitian adalah 50 μg/mL. Dengan demikian dilakukan pengenceran sebagai berikut.

V1 = 50/ 2000 x 1 mL = 0,025 mL = 25 μL ad. medium kultur 1000 μL

4. Pembuatan Larutan Uji Doxorubicin

Pembuatan larutan standar setiap konsentrasi mengikuti persamaan

C1 x V1 = C2 x V2

Ket : C1 = konsentrasi awal

V1 = volume awal

C2 = konsentrasi hasil pengenceran

V2 = volume larutan hasil pengenceran

Volume larutan hasil pengenceran yang ditetapkan untuk pengujian

adalah 400 μL.

Konsentrasi (C2)	**Volume ekstrak yang ditambahkan (V1)**	**Volume MK (ad 400 μL)**
0,5 μg/mL	4 μL	396 μL
1 μg/mL	8 μL	392 μL
20μg/mL	16 μL	384 μL
4 μg/mL	32 μL	368 μL
6 μg/mL	48 μL	352 μL
8 μg/mL	64 μL	336 μL
9 μg/mL	72 μL	328 μL
10 μg/mL	80 μL	320 μL
15 μg/mL	120 μL	280 μL

5. Data Absorbansi kultur sel MCF-7 pada 96 well-plate dengan perlakuan ekstrak etanol daun Prasman dan Doxorubicin.

No	Konsentrasi (μg/mL)	Absorbansi 1	Absorbansi 2	Absorbansi 3	Kontrol Sel	Kontrol Media
1	0	0,4437	0,4623	0,4606	0,5270	0,0526
2	50	0,4141	0,4258	0,3998	0,5156	0,0523
3	100	0,3714	0,4043	0,3714	0,5145	0,0487
4	200	0,3089	0,2927	0,2251		
5	300	0,2804	0,2464	0,2098		
6	400	0,2397	0,2068	0,1930		
7	500	0,2106	0,2394	0,2402		
8	600	0,1919	0,1492	0,1302		
9	800	0,5270	0,5156	0,5145		

No	Konsentrasi (μg/mL)	Absorbansi 1	Absorbansi 2	Absorbansi 3	Kontrol Sel	Kontrol Media
1	0	0,2253	0,2170	0,2094	0,2723	0,0526
2	0,5	0,1927	0,1763	0,1669	0,2782	0,0523

3	1	0,1620	0,1575	0,1519	0,2611	0,0487
4	2	0,1526	0,1521	0,1580		
5	4	0,1448	0,1375	0,1493		
6	6	0,1178	0,1107	0,1095		
7	8	0,0998	0,0987	0,0985		
8	9	0,0969	0,0965	0,0905		
9	10	0,0854	0,0853	0,0805		
10	15	0,2723	0,2782	0,2611		

6. Hasil Uji Statistik non regresi linier antara log konsentrasi ekstrak etanol daun Prasman dan viabilitas sel MCF-7.

log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	2.303
HillSlope	-1.568
IC50	201.0
95% CI (profile likelihood)	
LogIC50	2.231 to 2.368
HillSlope	-1.983 to -1.252
IC50	170.4 to 233.3
Goodness of Fit	
Degrees of Freedom	25
R squared	0.9324
Sum of Squares	1981
Sy.x	8.901
Number of points	
# of X values	27
# Y values analyzed	27

7. Hasil Uji Statistik Hasil Uji Statistik non regresi linier antara log konsentrasi Doxorubicin dan viabilitas sel MCF-7.

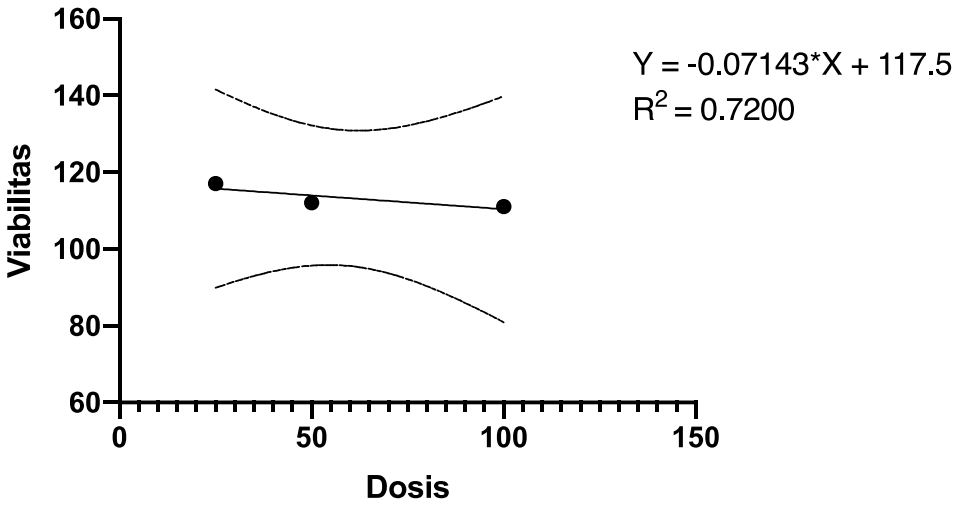
log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	0.2914
HillSlope	-1.187
IC50	1.956
95% CI (profile likelihood)	
LogIC50	0.1655 to 0.4076
HillSlope	-1.579 to -0.8840
IC50	1.464 to 2.556
Goodness of Fit	
Degrees of Freedom	28
R squared	0.7848
Sum of Squares	5865
Sy.x	14.47
Number of points	
# of X values	30
# Y values analyzed	30

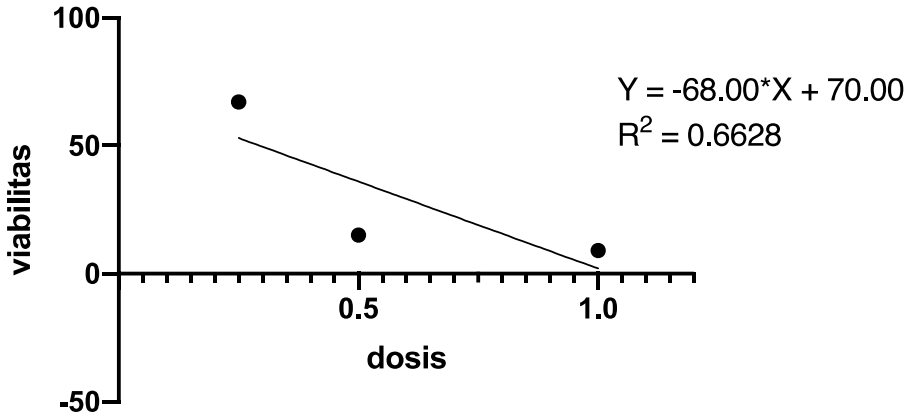
Lampiran. Perhitungan Uji sinergisitas Ekstrak Etanol Daun Prasman dan Doxorubicin

1. Nilai Absorbansi dan viabilitas sel

DOSIS		Absorbansi			Medium kultur	Viabilitas (%)
DOX	EEDP	1	2	3	Kontrol media	
0,25	25	0,3213	0,3271	0,3223	0,4453	**71,48%**
0,25	50	0,1501	0,1465	0,144	0,4139	**24,36%**
0,25	100	0,3226	0,2903	0,2584	0,4345	**62,53%**
0,5	25	0,0933	0,1129	0,107		**13,14%**
0,5	50	0,0831	0,0808	0,0785	0,0525	**6,76%**
0,5	100	0,1016	0,0879	0,0834	0,0581	**9,43%**
1	25	0,0735	0,0908	0,0948	0,0553	**8,32%**
1	50	0,1011	0,0769	0,0783		**7,91%**
1	100	0,1184	0,119	0,1216		**17,13%**
0,25		0,3117	0,2999	0,3136		**67,35%**
0,5		0,1103	0,1067	0,1167		**14,86%**
1		0,084	0,0924	0,089		**8,65%**
	25	0,5089	0,5059	0,4711		**117,23%**
	50	0,4869	0,4534	0,4834		**111,53%**
	100	0,5093	0,5073	0,3976		**110,94%**

2. Persamaan regresi linier ekstrak etanol daun Prasman dan Doxorubicin





3. Perhitungan Indeks Kombinasi

Dosis Ekstrak Etanol Daun Prasman

Dosis	25	50	100
0,25	648,17	1311,83	774,23
0,5	1469,86	1559,72	1522,11
1	1537,75	1543,52	1413,66

Dosis Doxorubicin

Dosis	**25**	**50**	**100**
0,25	-0,02	0,67	0,11
0,5	0,84	0,93	0,89
1	0,91	0,91	0,78

Indeks Kombinasi (IK)

Dosis	25	50	100
0,25	12,5	0,4	2,4
0,5	0,6	0,6	0,6
1	1,1	1,1	1,4

Lampiran. Ekspresi mRNA Bcl-2 dan Bax

1. Hasil perhitungan ekspresi relatif gen

Ekspresi mRNA	Perlakuan	Konsentrasi (µg/mL)	ΔCttest	ΔCtkontrol	ΔΔCt	Rasio ekspresi ($2^{-\Delta\Delta Ct}$)
Bax	EEDP	100	-0,53	-1,00	0,47	0,72
			-2,70	-1,31	-1,39	2,62
			-1,62	-0,25	-1,37	2,58
		200	0,47		1,47	0,36
			0,00		1,30	0,40
			-1,77		-1,53	2,80
	DOX	1	-5,01		-4,01	16,00
			-1,34		-0,03	1,02
			-4,40		-4,15	0,71
		2	-5,77		-4,77	25,90
			-7,65		-6,35	18,20
			-3,69		-3,44	10,50
Bcl-2	EEDP	100	3,18		4,18	0,06
			0,59		1,90	0,26
			1,88		2,13	0,22
		200	0,11		1,11	0,46
			3,45		4,75	0,03
			2,64		2,88	0,13
	DOX	1	-3,47		-2,47	0,25
			-1,45		-0,14	1,10

			-4,01		-3,76	13,54
		2	-5,40		-4,40	21,00
			-5,23		-3,93	14,90
			-6,02		-5,77	51,90