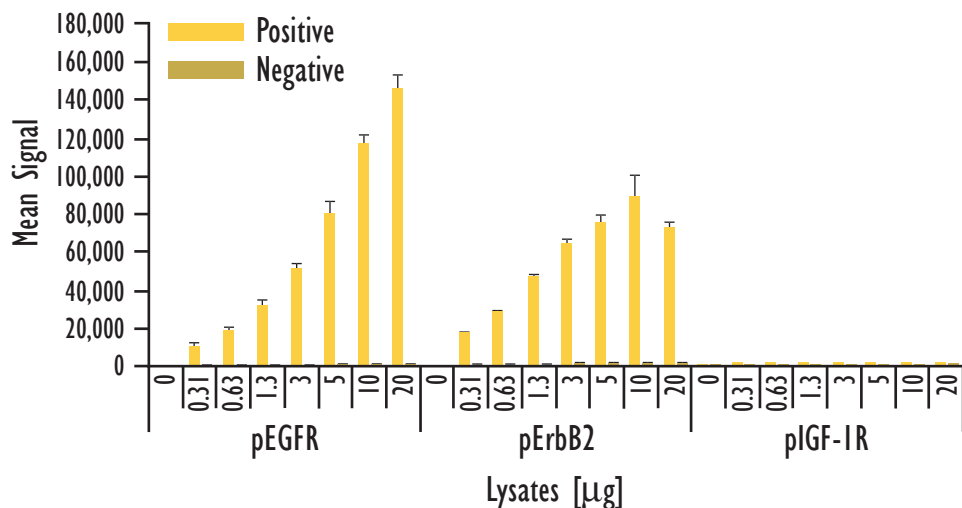
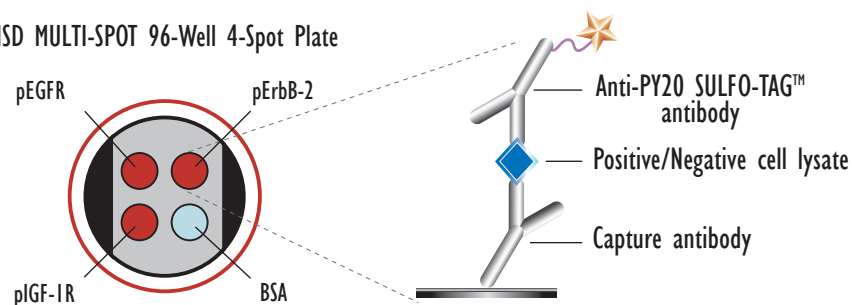


# MULTI-SPOT® EGFR Family Phosphorylation Assay

Multiplexed Detection of Phospho-EGFR, Phospho-ErbB-2 and Phospho-IGF-1R using PY20 Detection



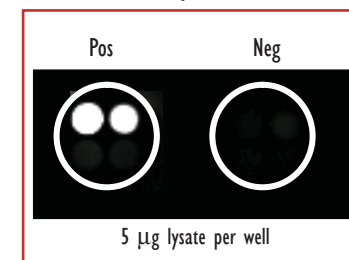
MSD MULTI-SPOT 96-Well 4-Spot Plate



	Lysates (µg)	Positive			Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pEGFR	0	42	13	31	31	4	13	
	0.31	10,642	1,499	14	163	12	8	65.3
	0.63	18,310	1,484	8	195	24	12	93.9
	1.3	32,041	2,716	8	228	5	2	140.5
	3	50,856	3,079	6	306	26	9	166.4
	5	79,822	6,244	8	405	24	6	197.3
	10	116,446	5,505	5	593	16	3	196.3
	20	145,766	6,947	5	763	33	4	191.0
pErbB2	0	45	12	26	41	12	30	
	0.31	16,931	292	2	470	17	4	36.0
	0.63	28,313	649	2	745	38	5	38.0
	1.3	46,384	1,801	4	986	15	2	47.0
	3	63,666	2,987	5	1,252	30	2	50.9
	5	74,933	4,654	6	1,390	59	4	53.9
	10	88,879	11,326	13	1,391	195	14	63.9
	20	71,859	3,649	5	1,345	67	5	53.4
pIGF-1R	0	43	7	16	40	10	25	
	0.31	487	17	3	125	6	5	3.9
	0.63	593	22	4	137	18	13	4.3
	1.3	673	13	2	150	24	16	4.5
	3	757	9	1	169	22	13	4.5
	5	778	26	3	189	7	4	4.1
	10	793	33	4	203	13	6	3.9
	20	797	16	2	221	12	5	3.6

Serum deprived SK-OV3 cells were treated with EGF (100 ng/mL; 10 min.) after a sodium vanadate pretreatment (1 mM; 4 hr)(positive) or Compound 56 (1 µM; 2.5 hr) and AEG825 (1 µM; 2.5 hr)(negative). Whole cell lysates were added to MSD MULTI-SPOT 4 Spot plates coated with anti-total-EGFR, anti-total ErbB-2, and anti-total IGF-1R antibodies on three of the four spatially distinct electrodes per well. Phosphorylated EGFR, ErbB-2, and IGF-1R were detected with anti-phosphotyrosine antibody labeled with MSD SULFO-TAG reagent.

MSD Experimental



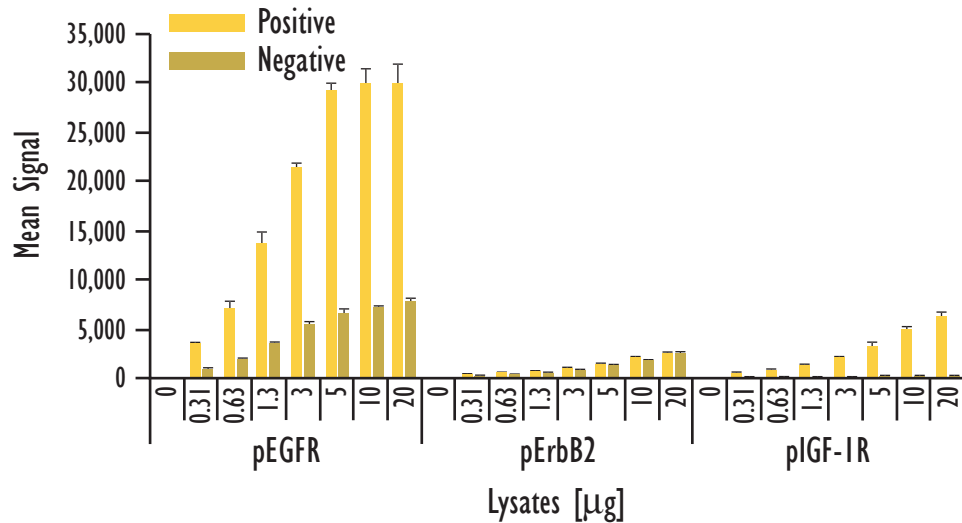
Kit Size	SI2400	SI6000
1 plate	KI5106D-1	KI1106D-1
5 plates	KI5106D-2	KI1106D-2
20 plates	KI5106D-3	KI1106D-3
20 plates (Base)	KI5106A-3	KI1106A-3



Meso Scale Discovery  
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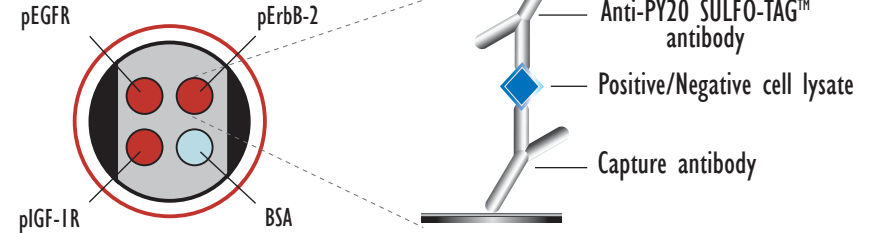
# MULTI-SPOT® EGFR Family Phosphorylation Assay

Multiplexed Detection of Phospho-EGFR, Phospho-ErbB-2 and Phospho-IGF-1R using PY20 Detection



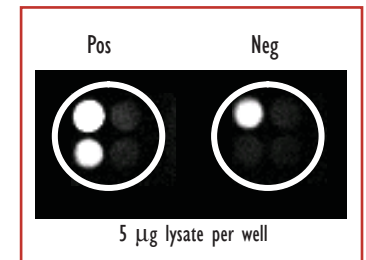
	Lysates (µg)	Positive			Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pEGFR	0	33	13	40	32	11	34	
	0.31	3,485	268	8	1,001	98	10	3.5
	0.63	7,109	692	10	2,005	140	7	3.5
	1.3	13,602	1,361	10	3,577	195	5	3.8
	3	21,418	487	2	5,477	293	5	3.9
	5	29,153	909	3	6,553	466	7	4.4
	10	29,838	1,582	5	7,160	290	4	4.2
	20	29,854	2,166	7	7,727	491	6	3.9
pErbB2	0	51	15	30	43	8	19	
	0.31	432	21	5	288	11	4	1.5
	0.63	598	28	5	417	10	2	1.4
	1.3	806	34	4	592	44	7	1.4
	3	1,143	40	4	882	37	4	1.3
	5	1,508	26	2	1,302	67	5	1.2
	10	2,122	106	5	1,899	101	5	1.1
	20	2,594	61	2	2,518	154	6	1.0
pIGF-1R	0	49	12	24	48	16	32	
	0.31	648	23	4	160	7	5	4.1
	0.63	967	59	6	184	7	4	5.3
	1.3	1,352	105	8	199	16	8	6.8
	3	2,135	58	3	223	9	4	9.6
	5	3,236	457	14	241	10	4	13.4
	10	5,003	322	6	247	11	4	20.3
	20	6,254	565	9	284	15	5	22.0

MSD MULTI-SPOT 96-Well 4-Spot Plate



Serum-deprived A431 cells (negative) were treated with IGF (100ng/ml; 10 min) after a sodium vanadate pretreatment (1mM; 4 hr) (positive). Whole-cell lysates were added to MSD MULTI-SPOT 4 Spot plates coated with anti-total EGFR, anti-total ErbB-2, and anti-total IGF-1R antibodies on three of the four spatially distinct electrodes per well. Phosphorylated EGFR, ErbB-2, and IGF-1R were detected with anti-phosphotyrosine antibody labeled with MSD SULFO-TAG reagent.

MSD Experimental



Kit Size	SI2400	SI6000
1 plate	KI5106D-1	KI1106D-1
5 plates	KI5106D-2	KI1106D-2
20 plates	KI5106D-3	KI1106D-3
20 plates (Base)	KI5106A-3	KI1106A-3