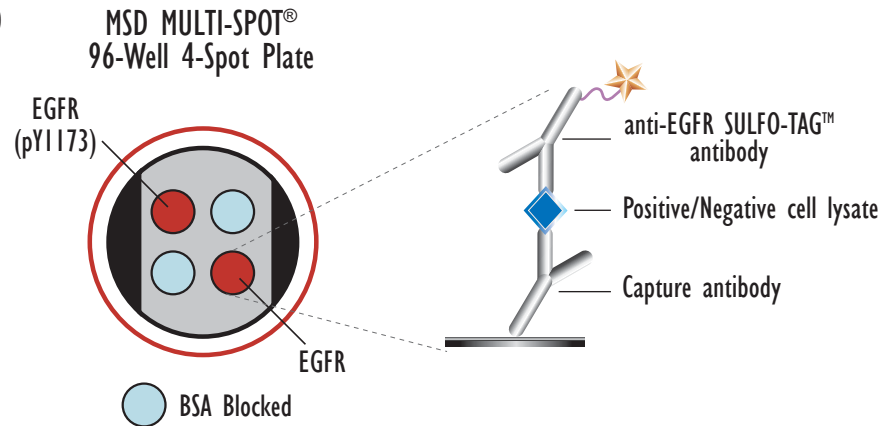
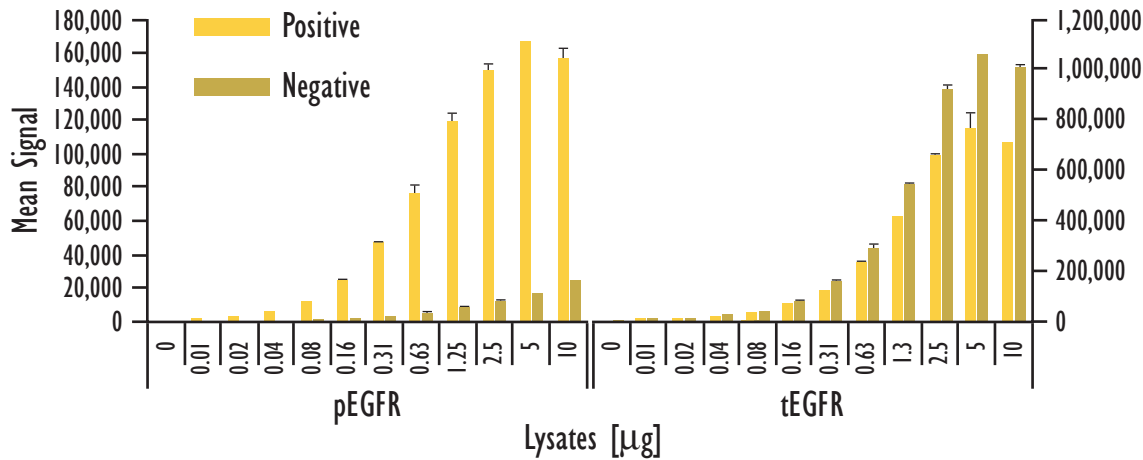


MULTI-SPOT[®] Phospho/Total EGFR (Tyr1173) Assay

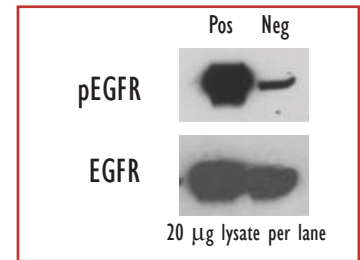
Detection of Total and Phosphorylated EGFR (Tyr1173) in Whole Cell Lysate Samples



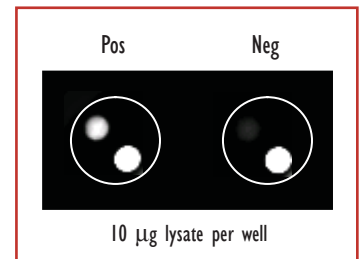
	Lysates (µg)	pEGFR Positive			pEGFR Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pEGFR	0				83	37	45	
	0.01	1,535	28	2	149	2	1	10.3
	0.02	3,093	272	9	242	11	5	12.8
	0.04	5,902	12	0	445	46	10	13.3
	0.08	11,857	193	2	913	53	6	13.0
	0.16	24,147	1,647	7	1,688	121	7	14.3
	0.31	47,152	573	1	3,174	29	1	14.9
	0.63	76,705	4,835	6	5,215	573	11	14.7
	1.25	118,913	4,691	4	8,261	431	5	14.4
	2.5	149,480	4,329	3	12,501	725	6	12.0
5	166,478	311	0	16,809	272	2	9.9	
10	156,688	6,253	4	24,320	398	2	6.4	
tEGFR	0				184	52	28	
	0.01	3,861	18	0	4,958	325	7	0.8
	0.02	7,637	180	2	9,914	670	7	0.8
	0.04	15,669	236	2	18,656	1,650	9	0.8
	0.08	30,948	817	3	39,656	276	1	0.8
	0.16	64,429	4,687	7	76,377	2,477	3	0.8
	0.31	121,570	292	0	157,668	5,238	3	0.8
	0.63	228,249	9,044	4	285,751	19,146	7	0.8
	1.25	413,168	675	0	544,371	2,655	0	0.8
	2.5	659,744	7,092	1	914,856	23,540	3	0.7
5	766,507	63,037	8	1,058,852	3,263	0	0.7	
10	711,199	1,256	0	1,006,322	14,843	1	0.7	

Serum-deprived A431 cells were treated with compound 56 (1 µM; 2.5 hr)(negative), or with EGF (100 ng/mL; 10 min)(positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-EGFR antibody and anti-total-EGFR antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total EGFR were detected with anti-total-EGFR antibody labeled with MSD SULFO-TAG reagent.

Traditional Western



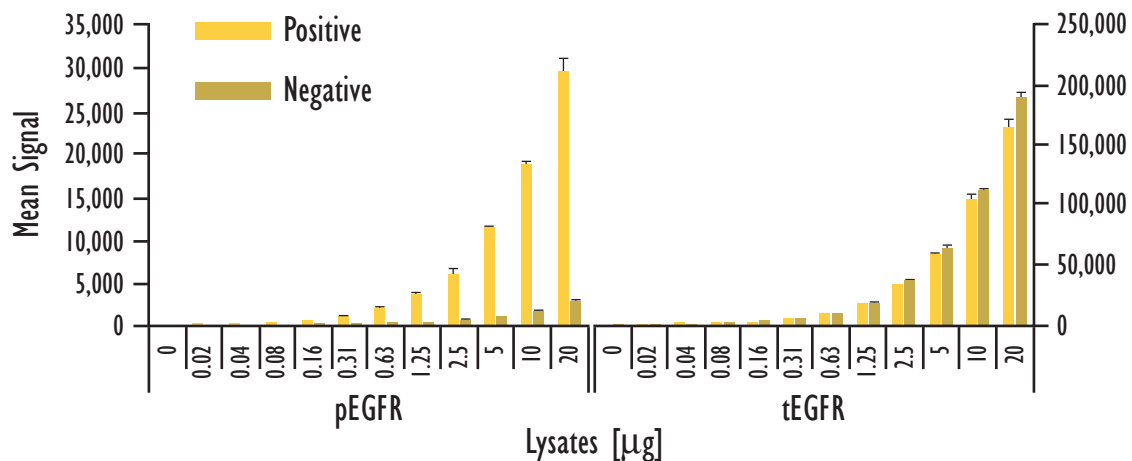
MSD Experimental



Kit Size	SI2400	SI6000
1 plate	K15104D-1	K11104D-1
5 plates	K15104D-2	K11104D-2
20 plates	K15104D-3	K11104D-3
20 plates (Base)	K15104A-3	K11104A-3

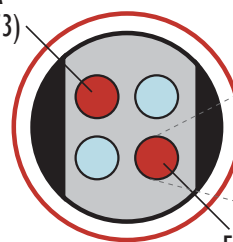
MULTI-SPOT[®] Phospho/Total EGFR (Tyr1173) Assay

Detection of Total and Phosphorylated EGFR (Tyr1173) in Whole Cell lysate Samples



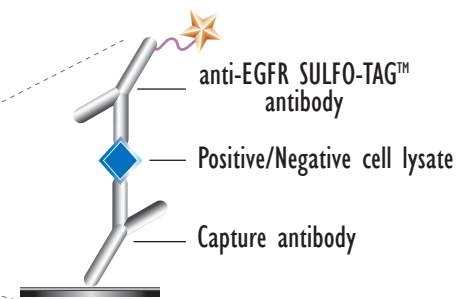
MSD MULTI-SPOT[®]
96-Well 4-Spot Plate

EGFR
(pY1173)



EGFR

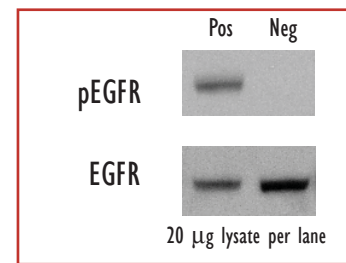
BSA Blocked



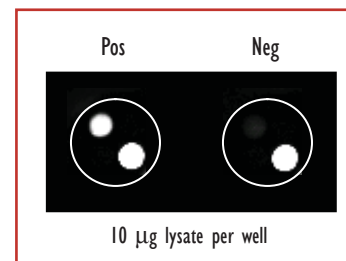
	Lysates (µg)	pEGFR Positive			pEGFR Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pEGFR	0				74	13	17	
	0.02	124	12	10	74	1	2	1.7
	0.04	226	23	10	81	8	10	2.8
	0.08	319	19	6	95	8	8	3.4
	0.16	606	6	1	161	6	4	3.8
	0.31	1,083	44	4	214	5	2	5.1
	0.63	2,068	119	6	311	32	10	6.7
	1.25	3,742	95	3	424	8	2	8.8
	2.5	5,986	717	12	705	40	6	8.5
	5	11,415	305	3	1,098	21	2	10.4
10	18,828	373	2	1,712	34	2	11.0	
20	29,591	1,651	6	2,935	38	1	10.1	
tEGFR	0				117	17	15	
	0.02	382	1	0	431	38	9	0.9
	0.04	1,046	536	51	700	9	1	1.5
	0.08	1,115	81	7	1,268	59	5	0.9
	0.16	2,199	111	5	2,489	115	5	0.9
	0.31	4,421	203	5	4,866	172	4	0.9
	0.63	8,729	93	1	9,086	222	2	1.0
	1.25	16,657	489	3	17,907	643	4	0.9
	2.5	32,427	1,176	4	35,960	1,232	3	0.9
	5	58,184	924	2	63,150	2,567	4	0.9
10	103,256	4,692	5	112,130	465	0	0.9	
20	164,260	7,383	4	188,770	4,751	3	0.9	

Serum-deprived COS-7 cells (negative) were treated with EGF (100 ng/mL, 10 min) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-EGFR antibody and anti-total-EGFR antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total EGFR were detected with anti-total-EGFR antibody labeled with MSD SULFO-TAG reagent.

Traditional Western



MSD Experimental



Kit Size	SI2400	SI6000
1 plate	K15104D-1	K11104D-1
5 plates	K15104D-2	K11104D-2
20 plates	K15104D-3	K11104D-3
20 plates (Base)	K15104A-3	K11104A-3



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