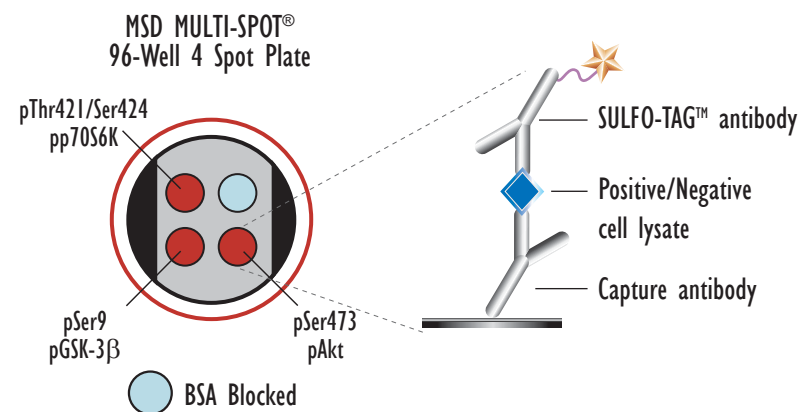
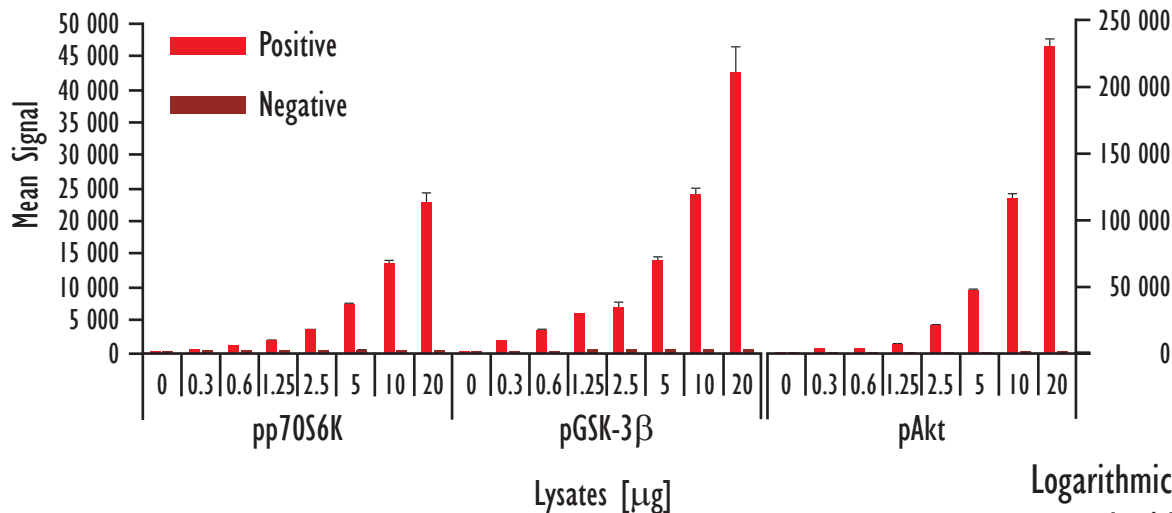


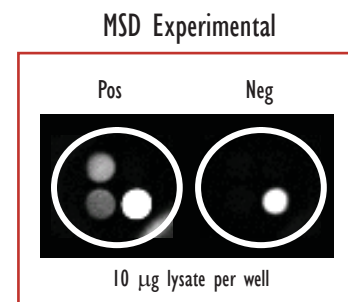
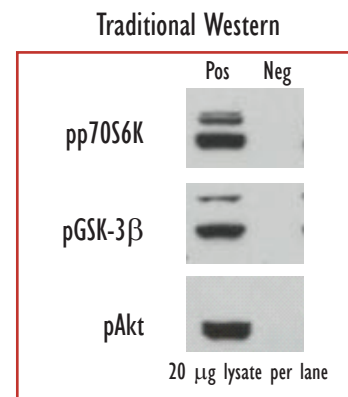
MULTI-SPOT[®] Akt Signaling Assay

Multiplexed Detection of Phosphorylated Akt (Ser473), p70S6K (Thr421/Ser424) and GSK-3 β (Ser9)



	Lysates (μ g)	Positive			Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pp70S6K	0	116	13	11	119	14	11	
	0.3	587	3	1	217	7	3	3
	0.6	1068	48	5	252	8	3	4
	1.25	1817	58	3	280	9	3	6
	2.5	3435	30	1	306	2	1	11
	5	7293	136	2	310	43	14	24
	10	13406	516	4	308	8	3	44
	20	22757	1230	5	306	14	5	74
pGSK-3 β	0	123	10	8	115	5	4	
	0.3	1833	30	2	243	3	1	8
	0.6	3442	54	2	298	3	1	12
	1.25	5965	194	3	362	30	8	16
	2.5	6852	817	12	373	2	0	18
	5	14072	447	3	393	34	9	36
	10	24022	1102	5	367	8	2	65
	20	42582	3916	9	393	7	2	108
pAkt	0	122	20	16	133	7	5	
	0.3	1476	24	2	243	14	6	6
	0.6	3079	40	1	293	18	6	10
	1.25	6848	161	2	370	9	3	19
	2.5	19833	991	5	404	45	11	49
	5	47091	480	1	447	18	4	105
	10	116205	2794	2	595	17	3	195
	20	229927	5742	2	893	14	2	258

Logarithmically growing Jurkat cells were treated with PMA (200 nM, 15min)(positive) or LY294002 (50 μ M) and staurosporine (1 μ M; 2.25hr)(negative). Whole cell lysates were added to MSD MULTI-SPOT 4 Spot plates coated with anti-total p70S6K, anti-phospho-GSK-3 β and anti-total Akt antibodies on three of the four spatially distinct electrodes per well. Phosphorylated p70S6K, GSK-3 β and Akt were detected with anti-phospho-p70S6K, anti-total GSK-3 β , and anti-phospho-Akt antibodies labeled with MSD SULFO-TAG reagent.



Kit Size	SI2400	SI6000
1 plate	K15115D-1	K11115D-1
5 plates	K15115D-2	K11115D-2
20 plates	K15115D-3	K11115D-3
20 plates (Base)	K15115A-3	K11115A-3