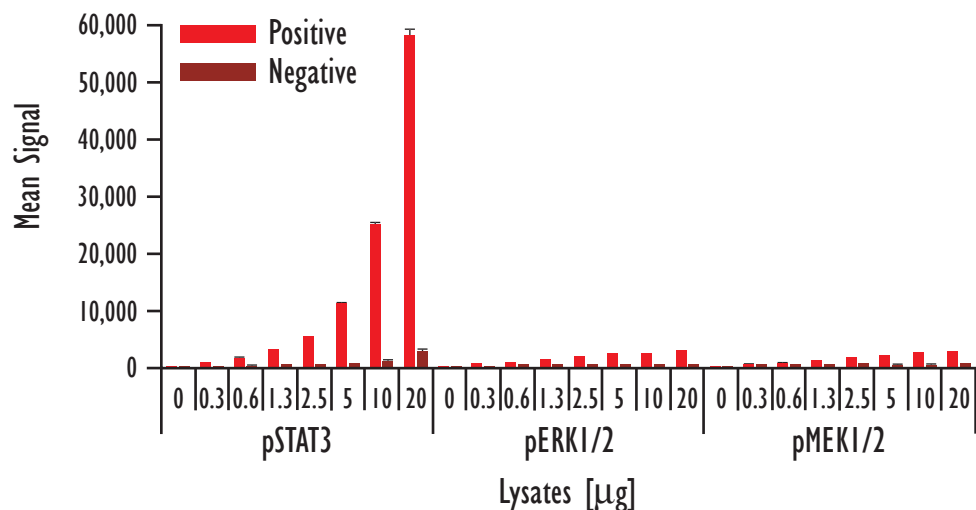
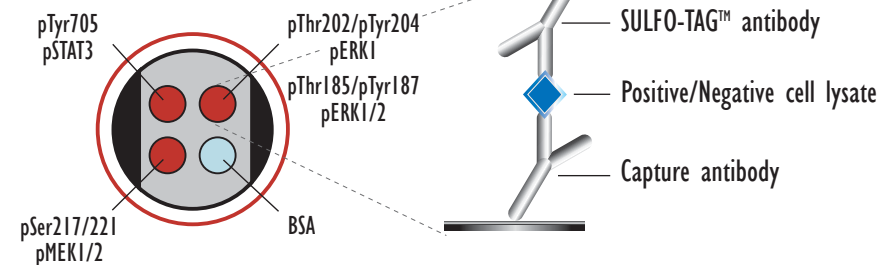


MULTI-SPOT[®] ERK-STAT3 Phosphorylation Cascade Assay

Detection of Phosphorylated STAT3, ERK1/2, and MEK1/2 in Whole Cell Lysate Samples



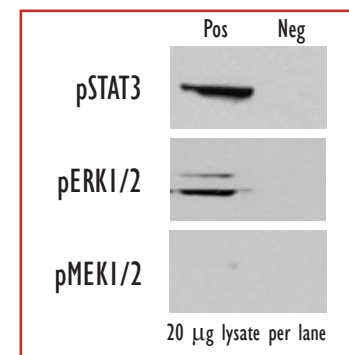
MSD MULTI-SPOT 96-Well 4-Spot Plate



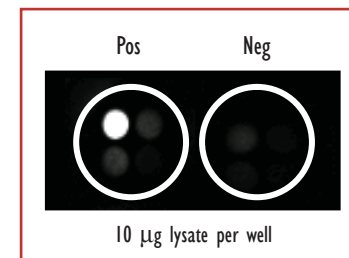
	Lysates (µg)	Positive			Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pSTAT3	0	133	14	10	137	16	11	
	0.3	982	22	2	256	35	14	3.8
	0.6	1,680	112	7	329	31	9	5.1
	1.3	3,012	51	2	441	22	5	6.8
	2.5	5,441	80	1	554	16	3	9.8
	5	11,131	252	2	730	54	7	15.3
	10	25,043	432	2	1,267	80	6	19.8
	20	58,005	1,379	2	2,935	329	11	19.8
pERK1/2	0	164	3	2	203	29	14	
	0.3	596	10	2	266	30	11	2.2
	0.6	906	23	3	345	27	8	2.6
	1.3	1,321	48	4	379	17	4	3.5
	2.5	1,868	152	8	427	23	5	4.4
	5	2,369	77	3	374	29	8	6.3
	10	2,335	147	6	321	54	17	7.3
	20	3,008	171	6	366	76	21	8.2
pMEK1/2	0	277	16	6	283	31	11	
	0.3	594	22	4	390	56	14	1.5
	0.6	823	28	3	454	48	11	1.8
	1.3	1,145	67	6	517	42	8	2.2
	2.5	1,674	44	3	607	34	6	2.8
	5	2,187	56	3	571	65	11	3.8
	10	2,538	97	4	529	73	14	4.8
	20	2,762	97	4	622	125	20	4.4

Confluent HeLa cells (negative) were treated with sodium vanadate (1 mM; 4 hr) and oncostatin M (40 ng/mL; 5 min)(positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-total-STAT3, anti-total-MEK1/2, and anti-phospho-ERK1/2 antibodies on three of the four spatially distinct electrodes per well. Phosphorylated STAT3, MEK1/2, and ERK1/2 were detected with anti-phospho-STAT3, anti-phospho-MEK1/2, and anti-total-ERK1/2 antibodies labeled with MSD SULFO-TAG reagent.

Traditional Western



MSD Experimental



Kit Size	SI2400	SI6000
1 plate	K15116D-1	K11116D-1
5 plates	K15116D-2	K11116D-2
20 plates	K15116D-3	K11116D-3
20 plates (Base)	K15116A-3	K11116A-3

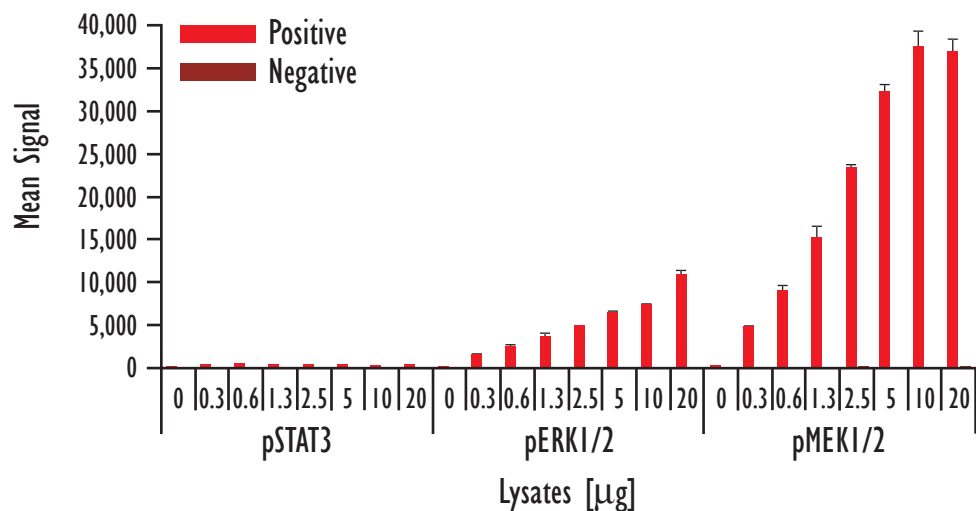


Meso Scale Discovery

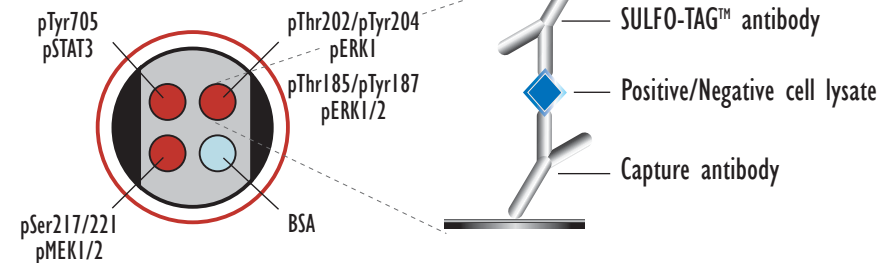
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MULTI-SPOT[®] ERK-STAT3 Phosphorylation Cascade Assay

Detection of Phosphorylated STAT3, ERK1/2, and MEK1/2 in Whole Cell Lysate Samples

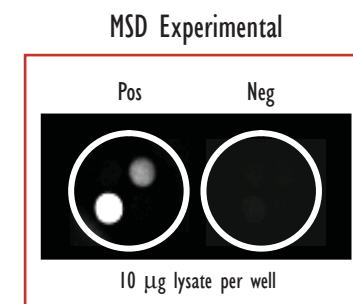
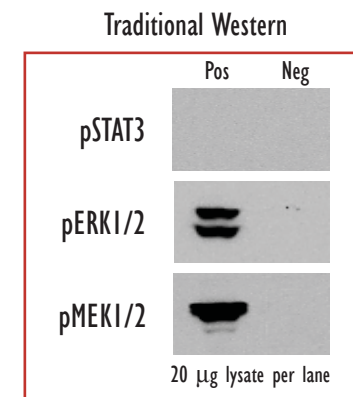


MSD MULTI-SPOT 96-Well 4-Spot Plate



	Lysates (µg)	Positive			Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pSTAT3	0	148	15	10	129	7	5	
	0.3	427	4	1	377	16	4	1.1
	0.6	442	12	3	400	4	1	1.1
	1.3	411	12	3	329	9	3	1.2
	2.5	360	26	7	276	9	3	1.3
	5	320	8	3	260	18	7	1.2
	10	301	22	7	295	34	11	1.0
	20	445	29	7	472	54	12	0.9
pERK1/2	0	188	8	4	180	38	21	
	0.3	1,543	92	6	396	27	7	3.9
	0.6	2,385	322	14	411	11	3	5.8
	1.3	3,600	448	12	363	44	12	9.9
	2.5	4,824	94	2	324	27	8	14.9
	5	6,411	214	3	276	34	12	23.2
	10	7,291	223	3	260	28	11	28.0
	20	10,821	589	5	303	38	12	35.7
pMEK1/2	0	301	10	3	292	15	5	
	0.3	4,729	191	4	601	10	2	7.9
	0.6	8,886	789	9	624	10	2	14.2
	1.3	15,206	1,321	9	558	41	7	27.3
	2.5	23,298	388	2	543	61	11	42.9
	5	32,231	880	3	579	28	5	55.6
	10	37,475	1,864	5	634	50	8	59.1
	20	36,868	1,531	4	659	103	16	55.9

Jurkat cells were treated with LY294002 (50 µM; 2.5 hr)(negative) or with PMA (200 nM; 15 min) (positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-total-STAT3, anti-total-MEK1/2, and anti-phospho-ERK1/2 antibodies on three of the four spatially distinct electrodes per well. Phosphorylated STAT3, MEK1/2, and ERK1/2 were detected with anti-phospho-STAT3, anti-phospho-MEK1/2, and anti-total-ERK1/2 antibodies labeled with MSD SULFO-TAG reagent.



Kit Size	SI2400	SI6000
1 plate	K15116D-1	K11116D-1
5 plates	K15116D-2	K11116D-2
20 plates	K15116D-3	K11116D-3
20 plates (Base)	K15116A-3	K11116A-3