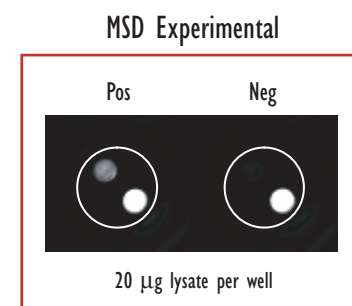
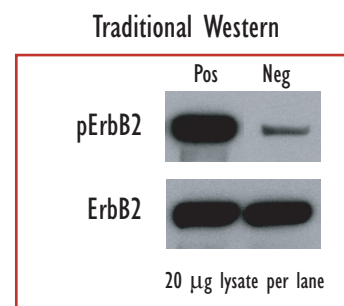
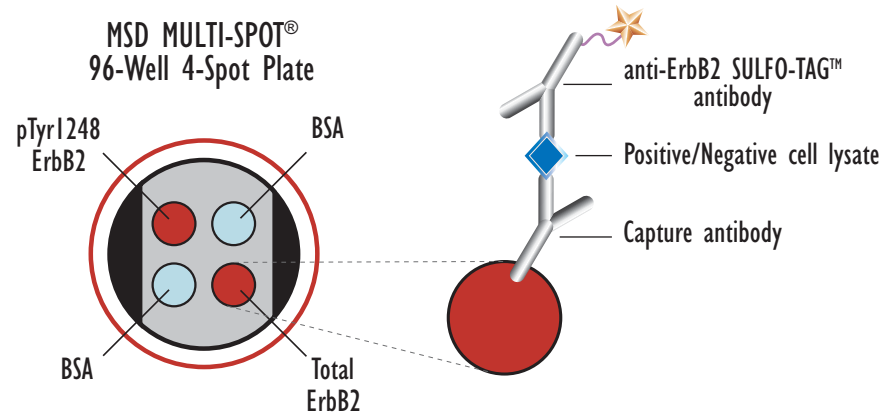
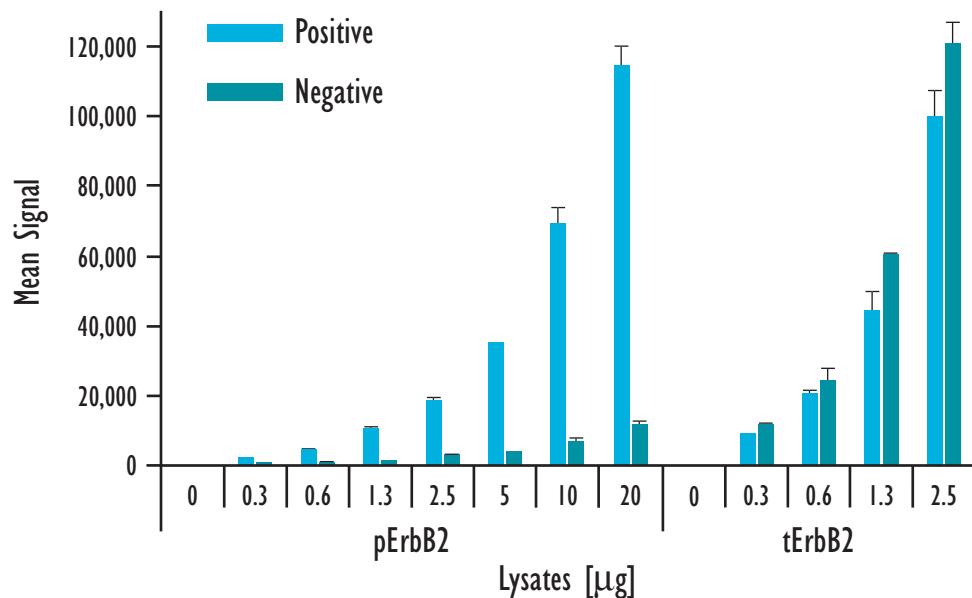


Multiplex ErbB2 Assay: Detection of Phosphorylated & Total ErbB2 in the Same Well



	Lysates (µg)	pErbB2 Positive			pErbB2 Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pErbB2	0	143	46	32	103	16	15	
	0.3	2,106	177	8	453	15	3	4.7
	0.6	4,325	420	10	699	87	12	6.2
	1.3	10,257	788	8	1,132	97	9	9.1
	2.5	18,123	1,404	8	2,472	432	17	7.3
	5	34,926	434	1	3,465	39	1	10.1
	10	69,222	4,781	7	6,945	1,071	15	10.0
	20	114,128	5,981	5	11,531	1,180	10	9.9
tErbB2	0	143	46	32	103	16	15	
	0.3	8,778	370	4	11,393	412	4	0.8
	0.6	20,624	730	4	24,101	3,479	14	0.9
	1.3	44,007	5,903	13	60,051	596	1	0.7
	2.5	99,453	8,015	8	120,799	5,893	5	0.8
	5	184,498	7,473	4	216,863	4,572	2	0.9
	10	300,929	7,352	2	340,928	8,338	2	0.9
	20	417,262	10,865	3	451,655	10,079	2	0.9

Serum deprived SK-OV3 cells were treated with orthovanadate (1 mM; 5 hr) followed by EGF stimulation (100 ng/mL; 10 min)(positive) or Compound 56 (1 µM; 3 hr) and AG825 (1 µM; 3 hr)(negative). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-ErbB2 antibody and anti-total-ErbB2 antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total ErbB2 were detected with anti-total-ErbB2 antibody labeled with MSD SULFO-TAG reagent.