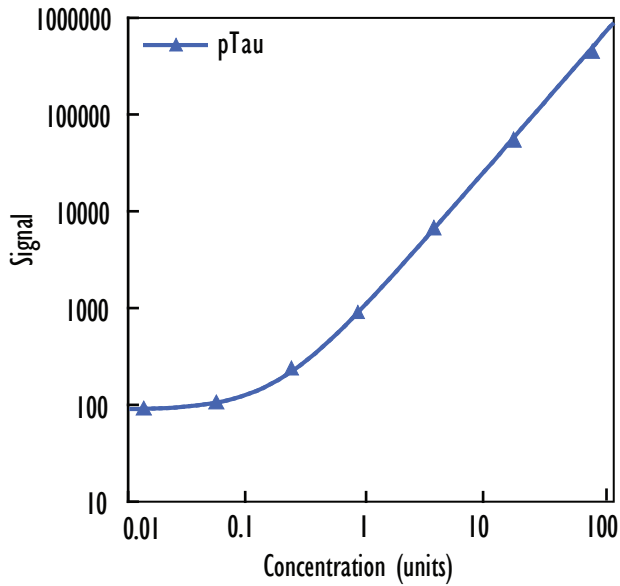


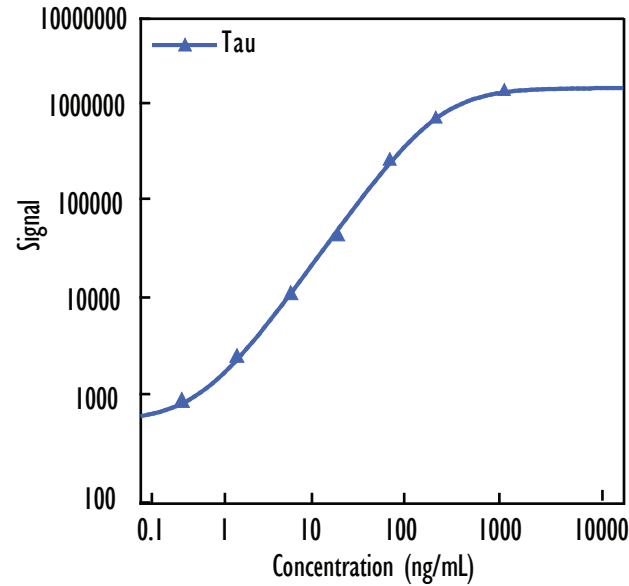
MULTI-SPOT[®] Phospho(Thr231)/Total Tau Assay

Phospho-Tau (Thr231)



pTau231 units/well	Average Signal	%CV
0	78	7.3
0.012	88	9.1
0.049	106	5.2
0.20	217	5.0
0.78	845	4.3
3.1	5384	10.8
13	38 996	4.0
50	326 001	3.5

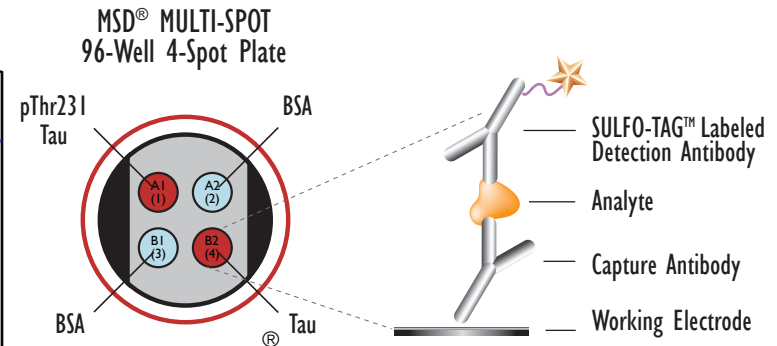
Total Tau



Total Tau(ng/mL)	Average Signal	%CV
0	483	4.4
0.24	852	1.8
0.98	2440	5.4
3.9	14 020	5.5
16	88 146	2.4
63	465 445	4.4
250	1 256 103	3.7
1000	1 561 670	2.0

Limit (or Limits) of Detection	
Phospho Tau (Thr231)	0.049 units*
Total Tau	0.076 ng/mL

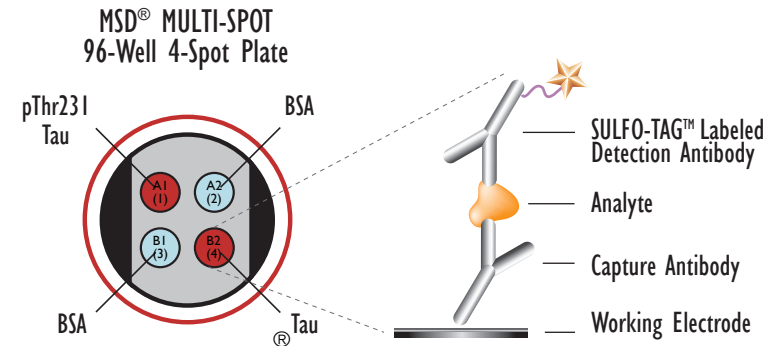
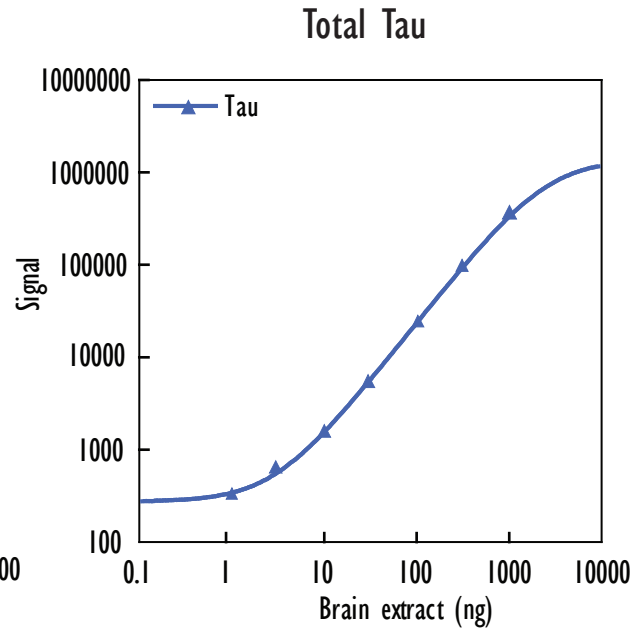
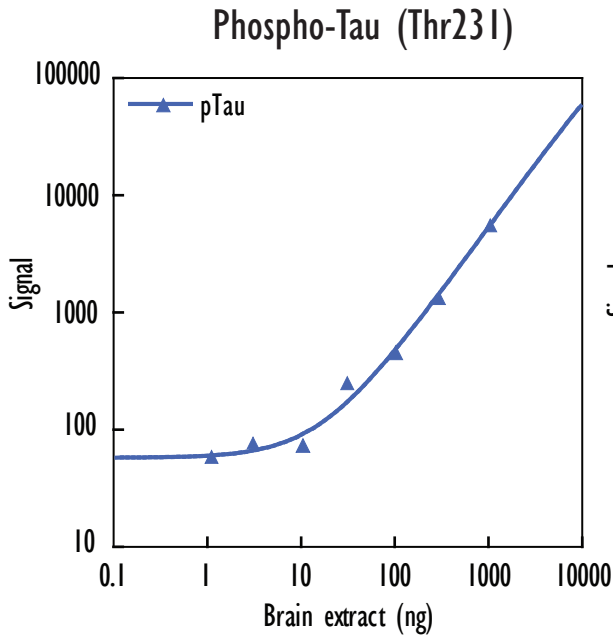
* Phosphorylated tau is quantified in units because the percentage of total tau that is phosphorylated will vary with different preparations of the standard.



Tau441 Calibrator (phosphorylated) was added to MSD MULTI-SPOT 4-spot, 96-well plates with one of the spatially distinct electrodes coated with anti-tau antibody and another coated with anti-phospho-tau antibody. Phosphorylated and total tau were detected with anti-tau conjugated with MSD SULFO-TAG reagent.

Kit Size	Catalog Number
1 plate	K15121D-1
5 plates	K15121D-2
20 plates	K15121D-3
20 plates (Base)	K15121A-3

Detection of Phosphorylated (Thr231) and Total Tau in Mouse Brain Extract

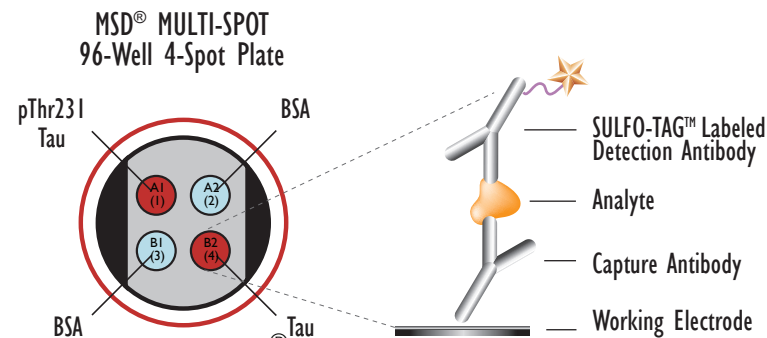
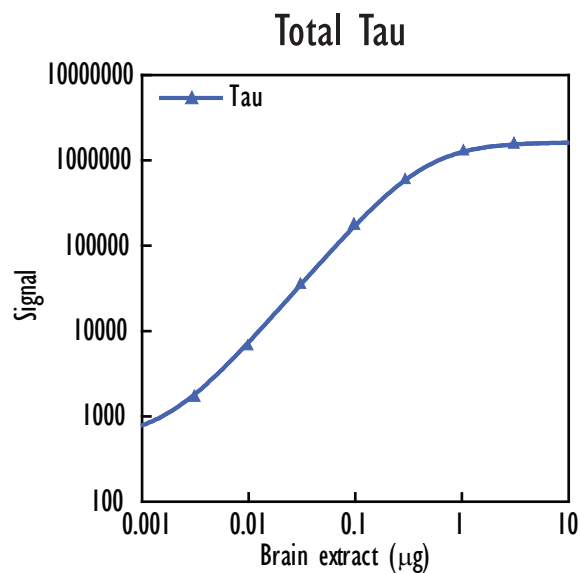
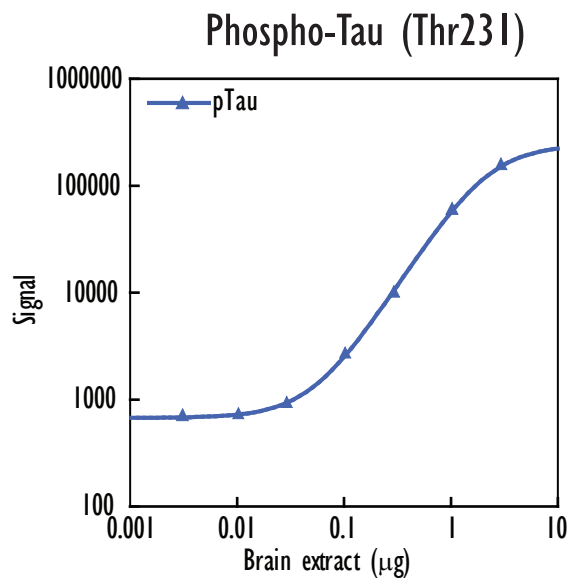


Mouse brain extract was prepared by homogenization of whole mouse brain in MSD Tris Lysis Buffer, followed by centrifugation to clear debris. Dilutions were prepared in MSD Tris Lysis Buffer. Samples were added to MSD MULTI-SPOT® 4-spot, 96-well plates with one of the spatially distinct electrodes coated with anti-tau antibody and another coated with anti-phospho-tau antibody. Phosphorylated and total tau were detected with anti-tau antibody conjugated with MSD SULFO-TAG reagent.

Extract (ng/well)	Phospho Tau (Thr231)			S/B
	Average Signal	StdDev	%CV	
0	56	19	33	
1.0	61	31	50	1
3.0	79	17	21	1
10	76	26	33	1
30	259	40	15	5
100	475	59	12	8
300	1380	166	12	25
1000	5726	680	12	102

Extract (ng/well)	Total Tau			S/B
	Average Signal	StdDev	%CV	
0	218	37	17	
1.0	337	16	5	2
3.0	654	45	7	3
10	1597	89	6	7
30	5519	512	9	25
100	24 256	1333	5	111
300	102 034	9042	9	467
1000	356 478	21 275	6	1633

Detection of Phosphorylated (Thr231) and Total Tau in Rhesus Brain Extract

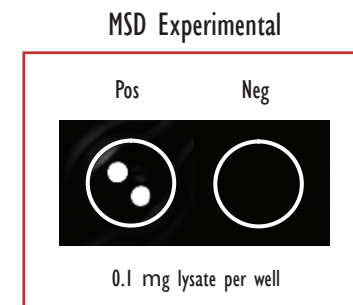
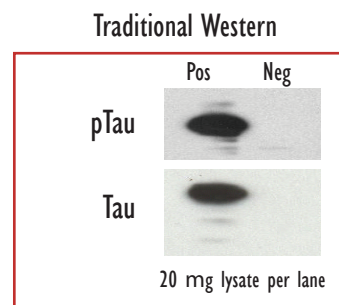
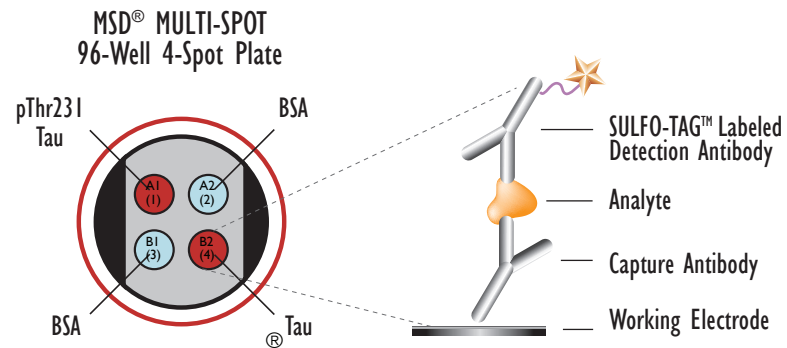
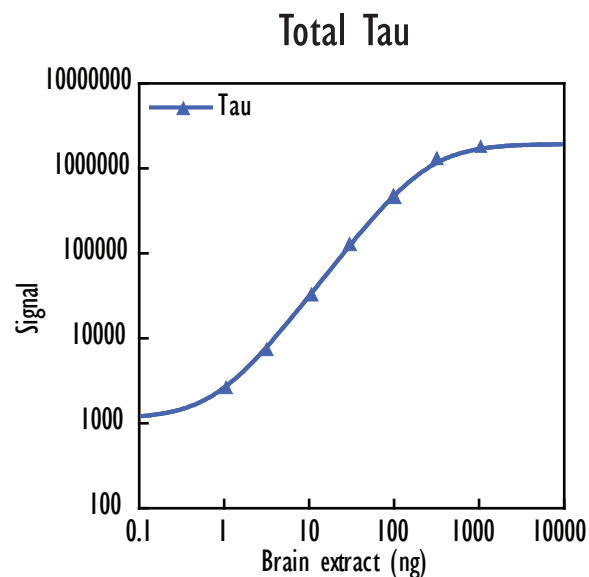
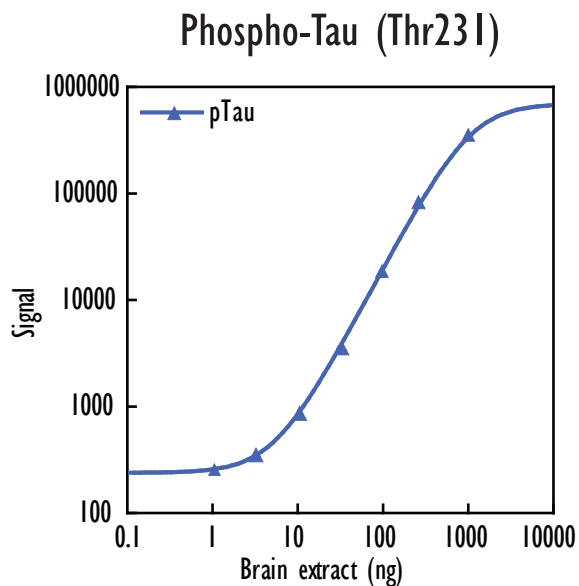


Rhesus brain extract was prepared by homogenization of rhesus brain tissue in MSD Tris Lysis Buffer, followed by centrifugation to clear debris. Dilutions were prepared in MSD Tris Lysis Buffer. Samples were added to MSD MULTI-SPOT® 4-spot, 96-well plates with one of the spatially distinct electrodes coated with anti-tau antibody and another coated with anti-phospho-tau antibody. Phosphorylated and total tau were detected with anti-rhesus tau antibody conjugated with MSD SULFO-TAG reagent.

Extract (µg/well)	Phospho Tau (Thr231)			S/B
	Average Signal	StdDev	%CV	
0	758	36	5	
0.003	750	34	5	1
0.01	767	42	5	1
0.03	929	75	8	1
0.1	2832	537	19	4
0.3	10530	1086	10	14
1.0	59382	2367	4	78
3.0	157825	6848	4	208

Extract (µg/well)	Total Tau			S/B
	Average Signal	StdDev	%CV	
0	442	42	9	
0.003	1830	130	7	4
0.01	7083	531	7	16
0.03	37746	1743	5	85
0.1	176684	4303	2	400
0.3	600636	23678	4	1358
1.0	1296457	47217	4	2932
3.0	1668563	91566	5	3773

Detection of Phosphorylated (Thr231) and Total Tau in Human Tau-441 Overexpressing CHO Cells



Extract (ng/well)	Phospho Tau (Thr231)			S/B
	Average Signal	StdDev	%CV	
0	214	14	7	
1.0	252	27	11	1
3.0	333	8	2	2
10	774	75	10	4
30	3621	344	9	17
100	18 233	812	4	85
300	87 296	5858	7	407
1,000	335 282	26 140	8	1564

Extract (ng/well)	Total Tau			S/B
	Average Signal	StdDev	%CV	
0	369	43	12	
1.0	2605	241	9	7
3.0	7911	29	0	21
10	30 304	3311	11	82
30	129 035	16 129	13	349
100	481 056	35 082	7	1303
300	1 218 344	62 380	5	3299
1,000	1 778 074	102 560	6	4814

CHO cells were transfected with a human tau-441 expression plasmid and harvested after 48 hours. Whole cell lysates were added to MSD MULTI-SPOT® 4-spot, 96-well plates with one of the spatially distinct electrodes coated with anti-tau antibody and another coated with anti-phospho-tau antibody. Phosphorylated and total tau were detected with anti-tau antibody conjugated with MSD SULFO-TAG reagent.