

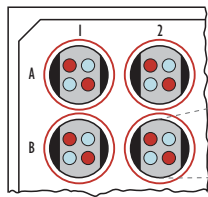
# Alzheimer's Research Products from MSD<sup>®</sup>



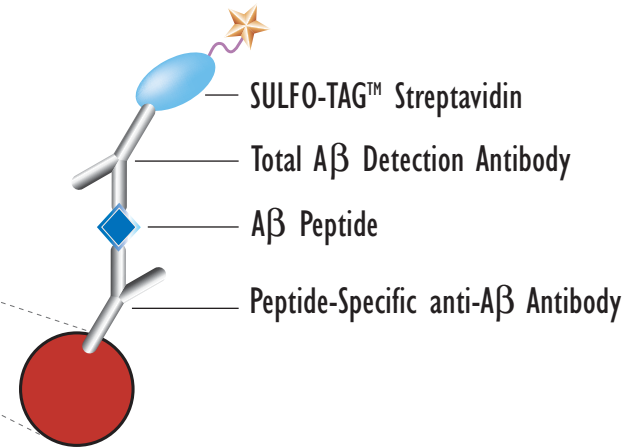
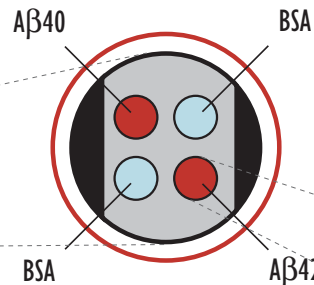
Meso Scale Discovery  
A division of Meso Scale Diagnostics, LLC.  
[www.mesoscale.com](http://www.mesoscale.com)

# Detection of A $\beta$ Peptides: $\beta$ -amyloid Peptide Duplex

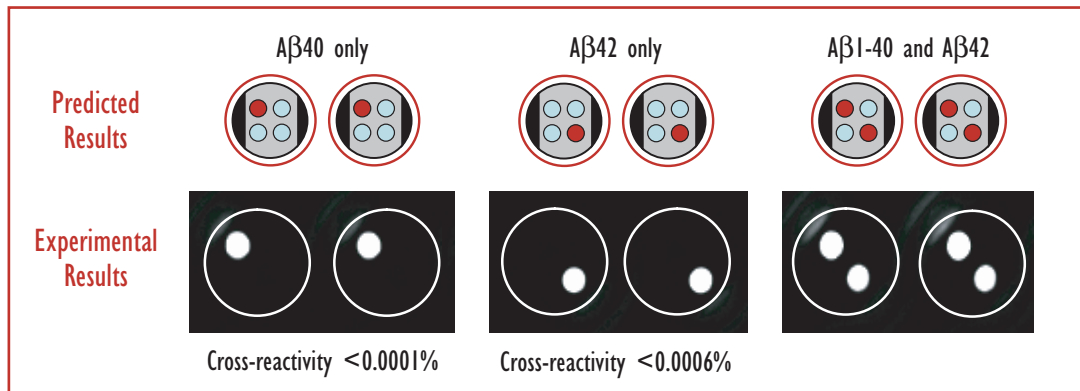
MSD MULTI-SPOT<sup>®</sup>  
96-Well 4-Spot Plate



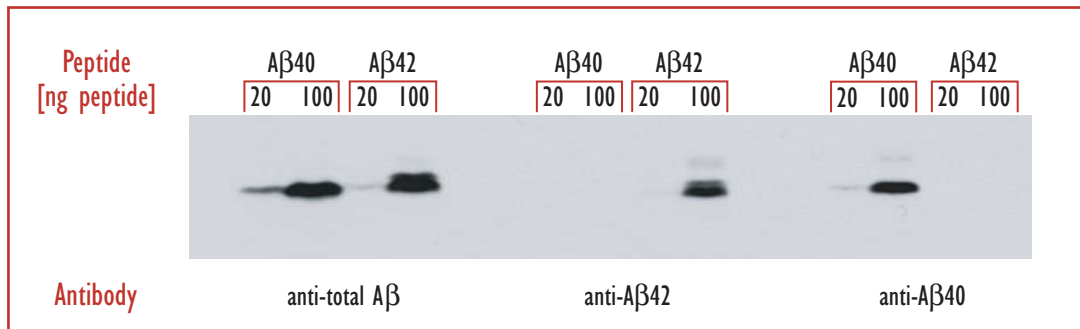
BSA



## MSD Experimental



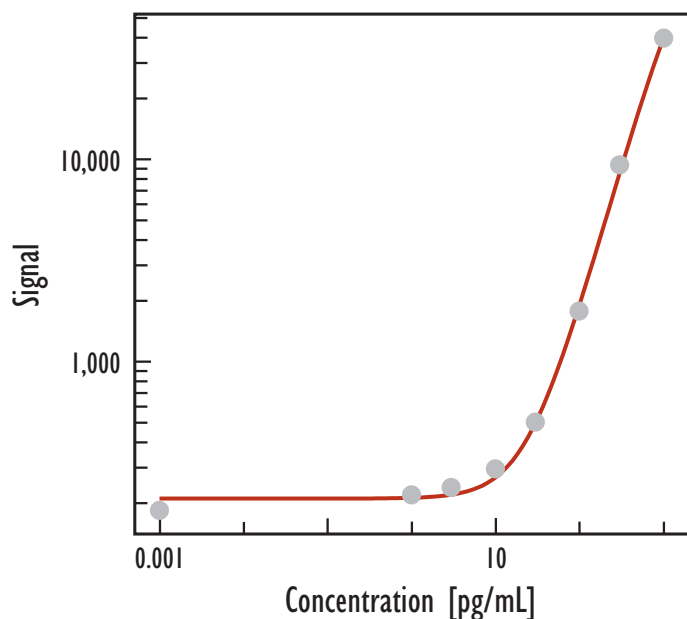
## Traditional Western



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# $\beta$ -amyloid Peptide Duplex in Lysis Buffer - 4G8 Detection

## A $\beta$ 40

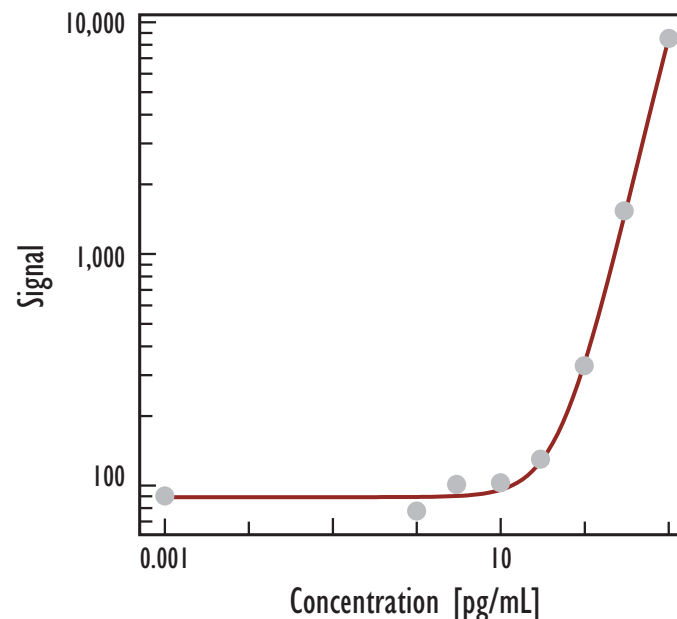


Peptide (pg/mL)	A $\beta$ 40			S/B
	Average	StdDev	%CV	
0	185	13	7	
1	221	19	9	1.2
3	239	20	8	1.3
10	296	19	6	1.6
30	506	19	4	2.7
100	1,771	152	9	9.6
300	9,394	922	10	50.9
1,000	39,601	2,159	5	214.6

**Detection Limit: 4 pg/mL**

Lysis Buffer: 150 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100

## A $\beta$ 42



Peptide (pg/mL)	A $\beta$ 42			S/B
	Average	StdDev	%CV	
0	90	9	10	
1	78	18	22	0.9
3	101	8	8	1.1
10	103	10	10	1.1
30	130	11	8	1.4
100	328	23	7	3.6
300	1,528	142	9	16.9
1,000	8,528	1,189	14	94.5

**Detection Limit: 26 pg/mL**

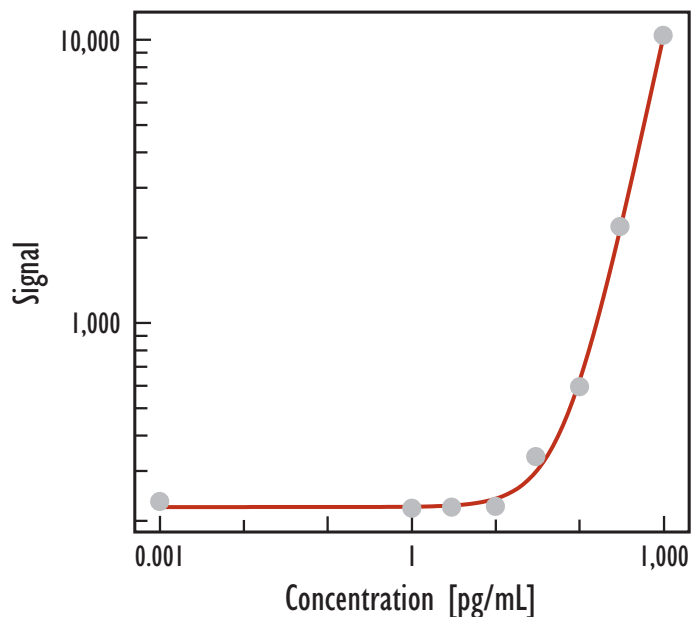
n=4; +/- StdDev Detection limit is 3 StdDev over 0 peptide



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# $\beta$ -amyloid Peptide Duplex in Culture Medium (10% FBS) - 4G8 Detection

## A $\beta$ 40

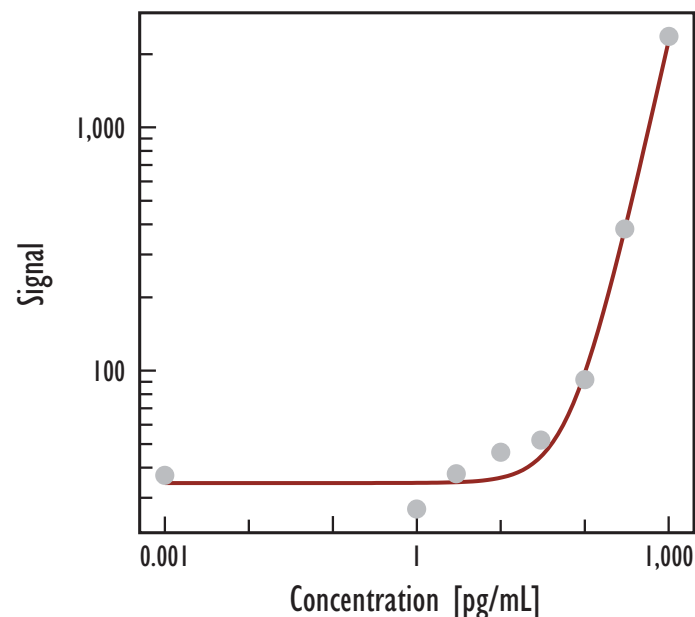


Peptide (pg/mL)	A $\beta$ 40			S/B
	Average	StdDev	%CV	
0	233	2	1	
1	221	10	5	0.9
3	223	25	11	1.0
10	225	29	13	1.0
30	336	6	2	1.4
100	594	224	38	2.5
300	2,180	35	2	9.3
1,000	10,362	609	6	44.4

**Detection Limit: 19 pg/mL**

Culture Medium: Iscove's medium with 10% fetal bovine serum and penicillin/streptomycin cocktail

## A $\beta$ 42



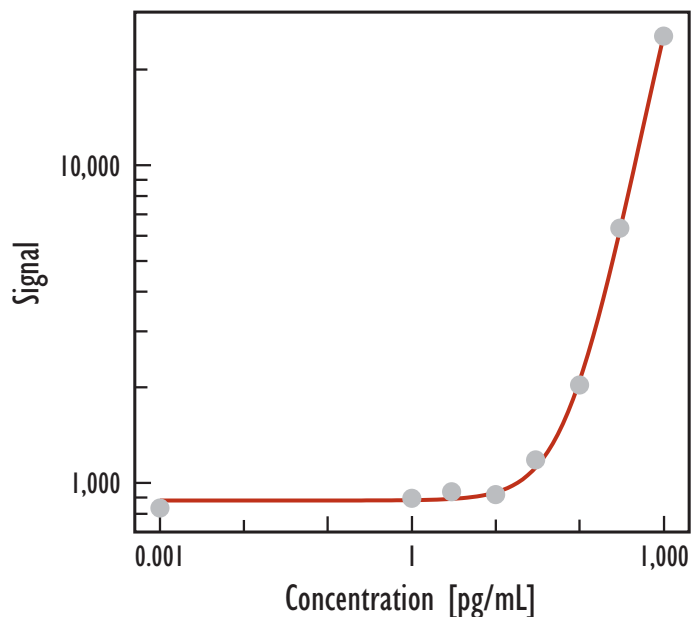
Peptide (pg/mL)	A $\beta$ 42			S/B
	Average	StdDev	%CV	
0	37	8	22	
1	27	6	23	0.7
3	38	5	13	1.0
10	46	5	10	1.2
30	52	13	24	1.4
100	92	22	24	2.5
300	381	50	13	10.3
1,000	2,373	174	7	64.1

**Detection Limit: 65 pg/mL**

n=4; +/- StdDev Detection limit is 3 StdDev over 0 peptide

# $\beta$ -amyloid Peptide Duplex in Culture Medium (10% FBS) - 6E10 Detection

## A $\beta$ 40

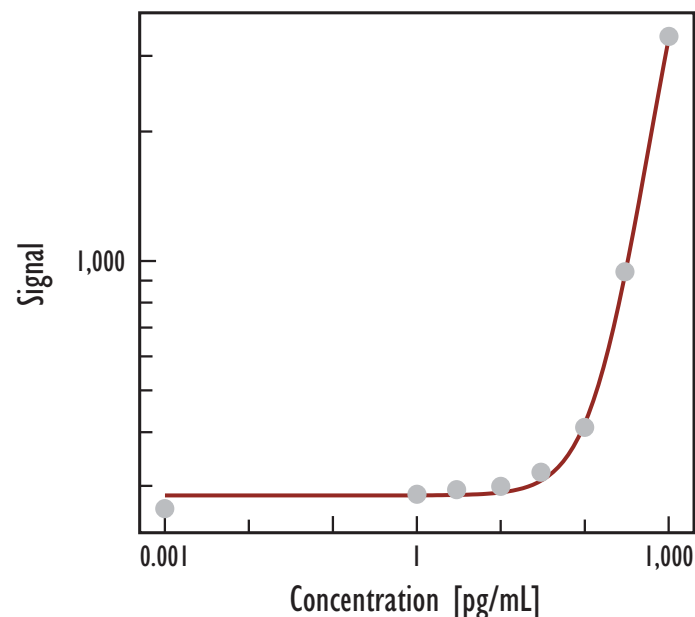


Peptide (pg/mL)	A $\beta$ 40			S/B
	Average	StdDev	%CV	
0	832	65	8	
1	892	85	10	1.1
3	937	63	7	1.1
10	919	51	6	1.1
30	1,178	53	5	1.4
100	2,027	112	6	2.4
300	6,328	233	4	7.6
1,000	25,468	1,512	6	30.6

**Detection Limit: 21 pg/mL**

Culture Medium: Iscove's medium with 10% fetal bovine serum and penicillin/streptomycin cocktail

## A $\beta$ 42



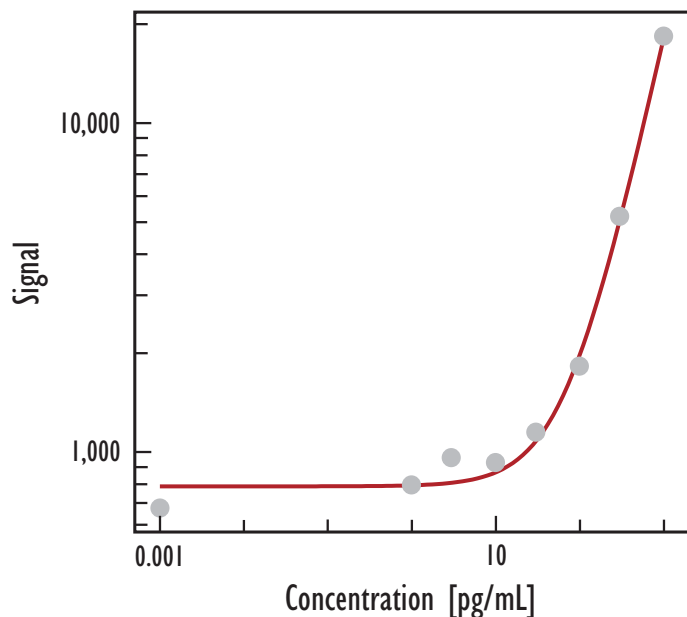
Peptide (pg/mL)	A $\beta$ 42			S/B
	Average	StdDev	%CV	
0	265	14	5	
1	287	19	7	1.1
3	293	23	8	1.1
10	299	10	3	1.1
30	322	21	6	1.2
100	409	22	5	1.5
300	942	19	2	3.6
1,000	3,322	176	5	12.5

**Detection Limit: 29 pg/mL**

n=4; +/- StdDev Detection limit is 3 StdDev over 0 peptide

# Detection of A $\beta$ Peptides: $\beta$ -amyloid Peptide Human Serum - 4G8 Detection

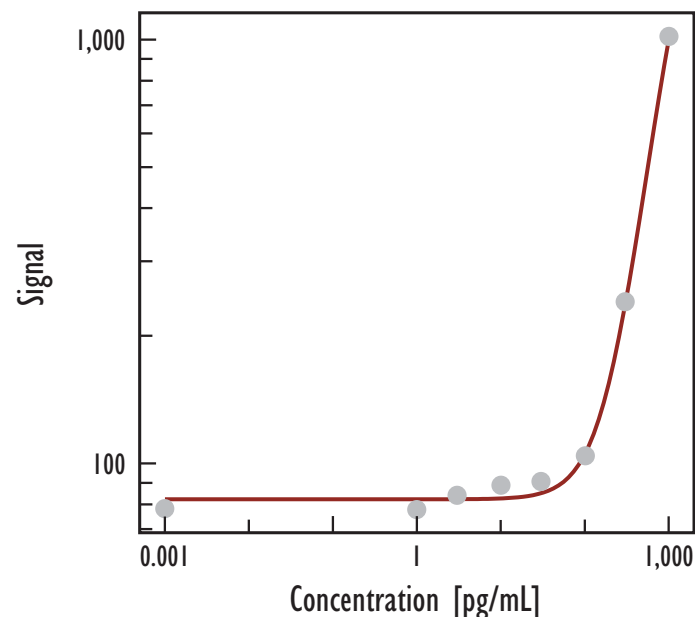
## A $\beta$ 40



Peptide (pg/mL)	A $\beta$ 40			S/B
	Average	StdDev	%CV	
0	716	64	9	
1	800	20	3	1.1
3	836	61	7	1.2
10	897	39	4	1.3
30	1,107	82	7	1.5
100	1,722	329	19	2.4
300	4,909	428	9	6.9
1,000	16,081	3,749	23	22.5

**Detection Limit: 12 pg/mL**

## A $\beta$ 42

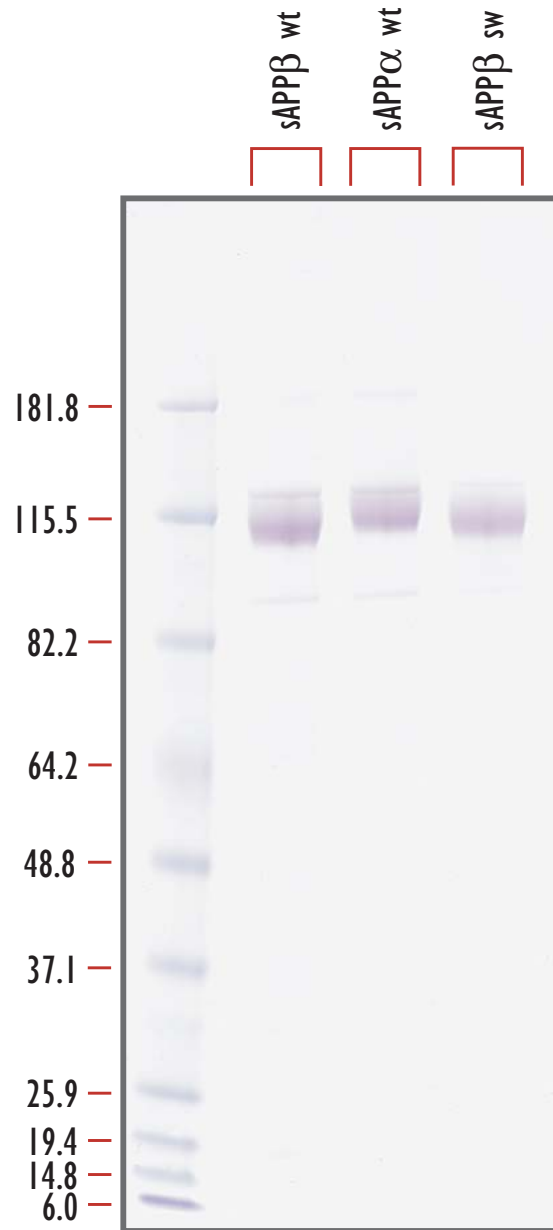


Peptide (pg/mL)	A $\beta$ 42			S/B
	Average	StdDev	%CV	
0	77	9	12	
1	73	17	23	1.0
3	77	16	20	1.0
10	85	9	11	1.1
30	82	13	16	1.1
100	95	20	21	1.2
300	228	27	12	3.0
1,000	965	164	17	12.5

**Detection Limit: 105 pg/mL**

100% non-sterile normal mouse serum n=4; +/- StdDev Detection limit is 3 StdDev over 0 peptide

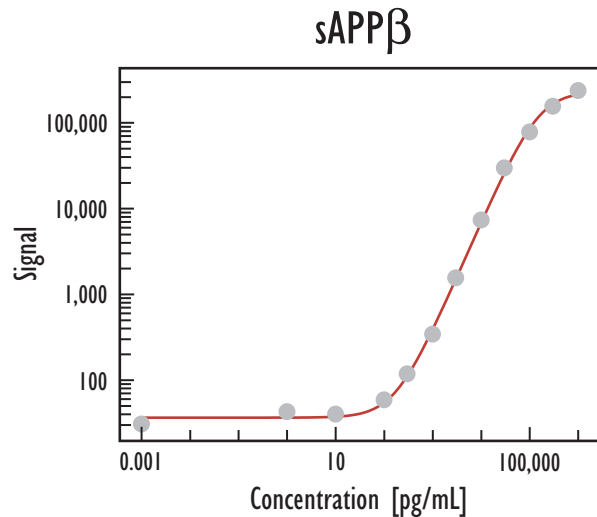
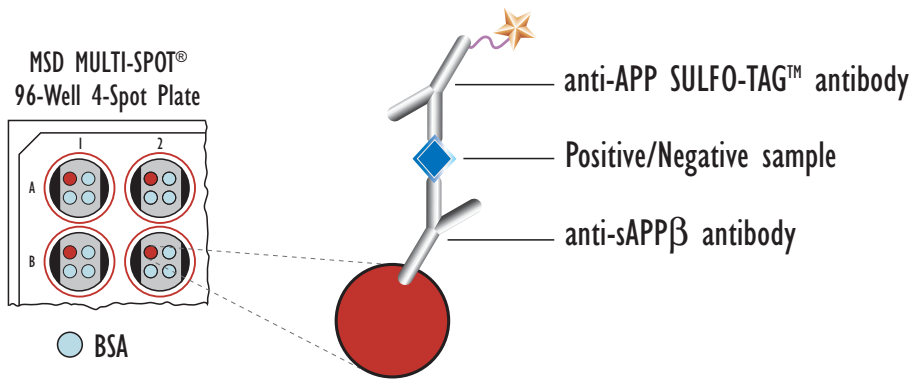
# Recombinant Human sAPP Protein Standards



Recombinant human sAPP proteins were purified from mammalian cells. A 0.5  $\mu$ g sample of each protein was run on 4-12% Bis-Tris NuPAGE gel to demonstrate purity (>95%).

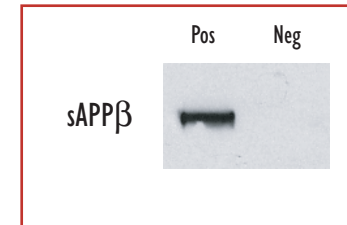


# Detection of Human Soluble APP $\beta$ (sAPP $\beta$ )

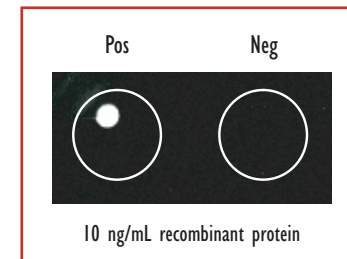


Concentration	Average	StdDev	%CV	P/N
0	31	15	49	
1 pg/mL	43	15	34	1
10 pg/mL	40	8	20	1
100 pg/mL	59	13	23	2
300 pg/mL	118	12	10	4
1 ng/mL	342	16	5	11
3 ng/mL	1,553	72	5	51
10 ng/mL	7,350	541	7	240
30 ng/mL	29,802	2,262	8	972
100 ng/mL	78,230	4,371	6	2,551
300 ng/mL	155,928	6,771	4	5,085
1 $\mu$ g/mL	237,557	7,991	3	7,746

Traditional Western



MSD Experimental



Recombinant human sAPP $\beta$  was purified from mammalian cells (>95% pure) and diluted in fresh culture medium (DMEM, 10% FBS, Pen/Strep). Samples were added to MSD MULTI-SPOT 4-Spot plates coated with anti-sAPP $\beta$  antibody on one of the four spatially distinct electrodes per well. The sAPP $\beta$  protein was detected with anti-APP antibody labeled with MSD SULFO-TAG reagent.

Cross Reactivity (at 100 ng/mL)

sw sAPP $\beta$   $\leq 0.05\%$

sAPP $\alpha$   $\leq 0.08\%$

wt sAPP $\beta$   
Detection Limit  
(3 StdDev over background)

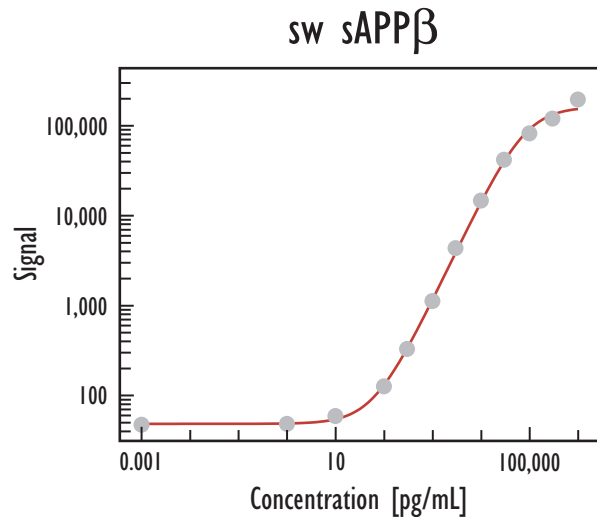
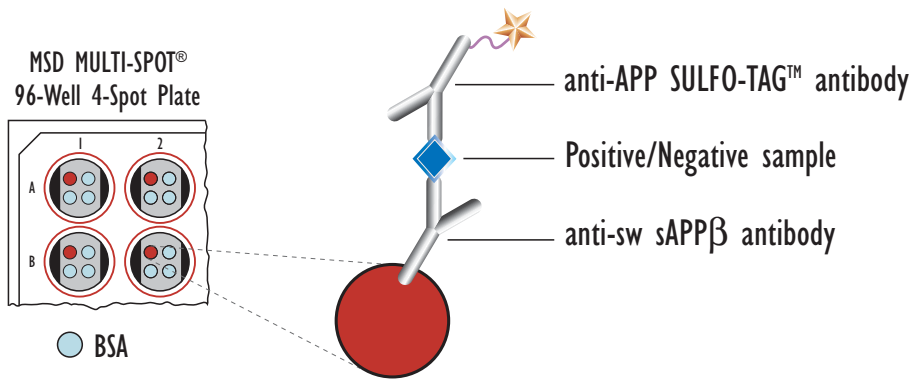
182 pg/mL



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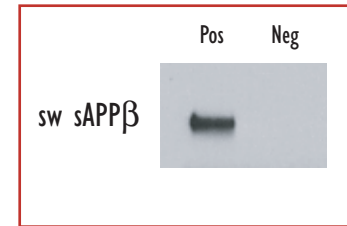


# Detection of Human Swedish Soluble APP $\beta$ (sw sAPP $\beta$ )

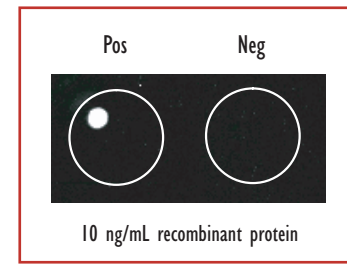


Concentration	Average	StdDev	%CV	P/N
0	47	9	19	
1 pg/mL	48	11	24	1
10 pg/mL	59	22	37	1
100 pg/mL	126	18	14	3
300 pg/mL	329	15	4	7
1 ng/mL	1,120	37	3	24
3 ng/mL	4,345	176	4	92
10 ng/mL	14,664	356	2	310
30 ng/mL	41,785	125	0	883
100 ng/mL	81,860	20,236	25	1,729
300 ng/mL	119,720	3,060	3	2,529
1 $\mu$ g/mL	194,794	19,973	10	4,115

Traditional Western



MSD Experimental



Recombinant human Swedish sAPP $\beta$  was purified from mammalian cells (>95% pure) and diluted in fresh culture medium (DMEM, 10% FBS, Pen/Strep). Samples were added to MSD MULTI-SPOT 4-Spot plates coated with anti-Swedish sAPP $\beta$  antibody on one of the four spatially distinct electrodes per well. The Swedish sAPP $\beta$  protein was detected with anti-APP antibody labeled with MSD SULFO-TAG reagent.

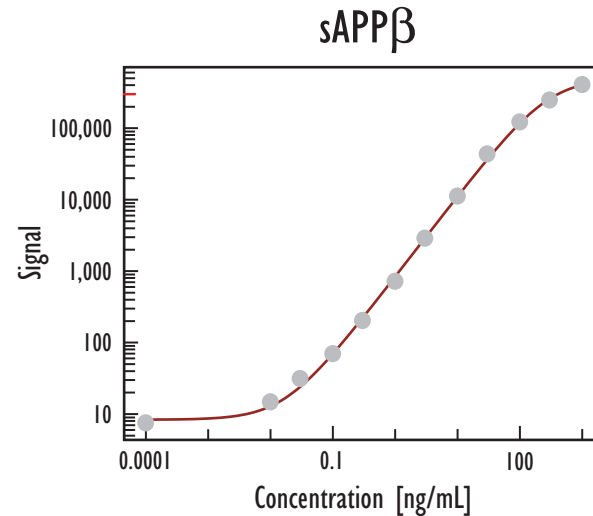
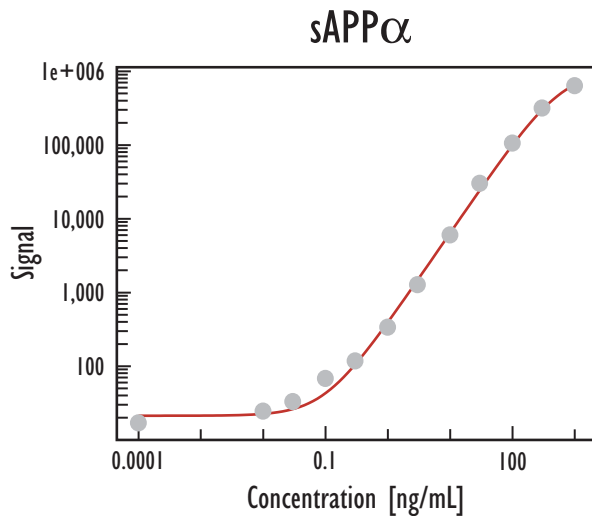
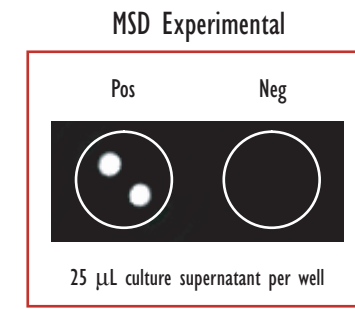
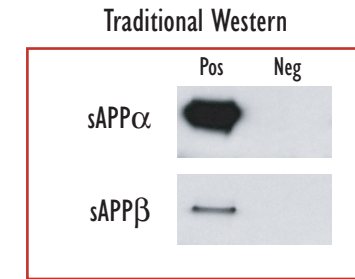
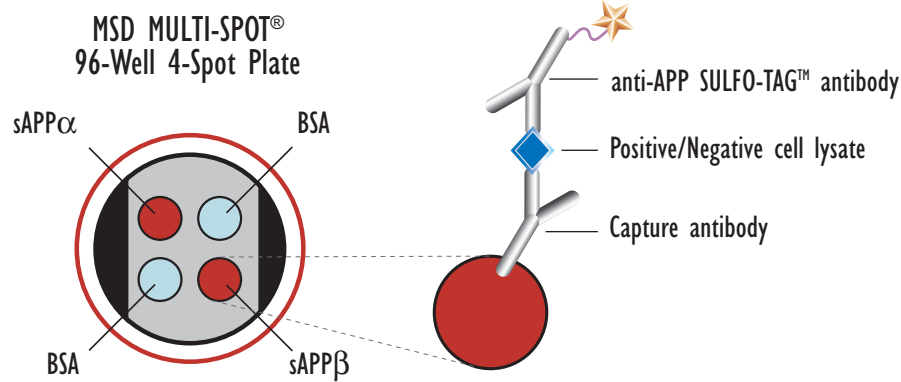
Cross Reactivity (at 100 ng/mL)

wt sAPP $\beta$   $\leq 0.07\%$

sAPP $\alpha$   $\leq 0.10\%$

sw sAPP $\beta$   
Detection Limit  
(3 StdDev over background) 39 pg/mL

# Multiplex Soluble APP Assay: Detection of Human sAPP $\alpha$ & sAPP $\beta$ in the Same Well



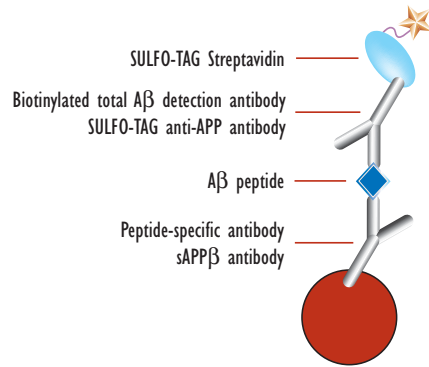
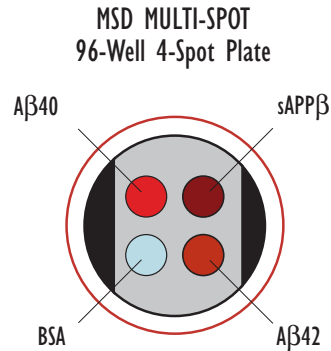
Concentration	Average	StdDev	%CV	P/N
0	17	10	62	1
0.01 ng/mL	32	9	28	2
0.03 ng/mL	33	7	22	2
0.1 ng/mL	68	8	12	4
0.3 ng/mL	118	7	6	7
1 ng/mL	337	3	1	20
3 ng/mL	1,271	49	4	75
10 ng/mL	6,012	586	10	354
30 ng/mL	30,119	491	2	1,772
100 ng/mL	105,764	17,363	16	6,221
300 ng/mL	316,441	6,268	2	18,614
1 $\mu$ g/mL	634,377	50,042	8	37,316

Concentration	Average	StdDev	%CV	P/N
0	10	6	62	1
0.01 ng/mL	20	6	31	2
0.03 ng/mL	31	5	16	4
0.1 ng/mL	70	14	20	9
0.3 ng/mL	203	18	9	26
1 ng/mL	720	60	8	93
3 ng/mL	2,878	219	8	371
10 ng/mL	11,193	1,596	14	1,444
30 ng/mL	43,550	3,390	8	5,619
100 ng/mL	121,938	5,558	5	15,734
300 ng/mL	246,786	16,891	7	31,843
1 $\mu$ g/mL	405,610	35,490	9	52,337

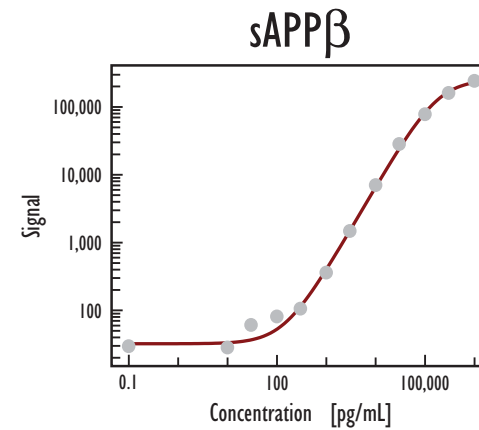
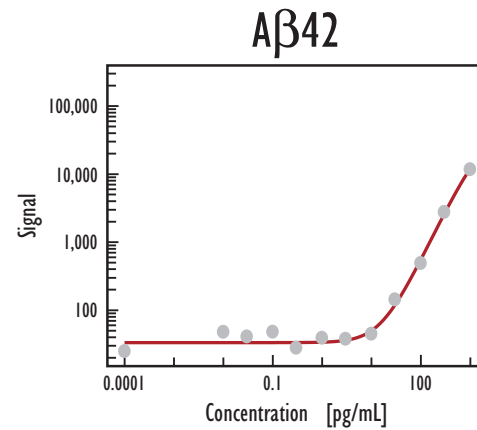
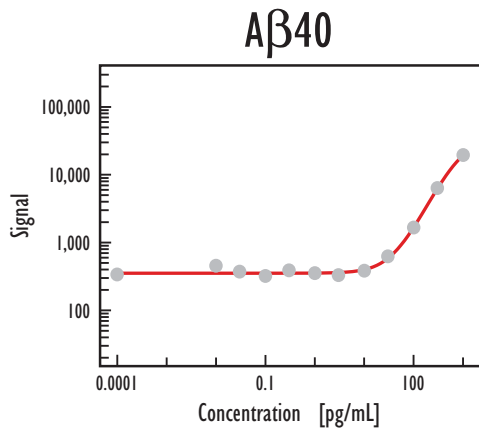
Recombinant human sAPP $\alpha$  and sAPP $\beta$  were purified from mammalian cells (>95% pure) and diluted in fresh culture medium (DMEM, 10% FBS, Pen/Strep). Samples were added to MSD MULTI-SPOT 4-Spot plates coated with anti-sAPP $\alpha$  and sAPP $\beta$  antibodies on two of the four spatially distinct electrodes per well. The sAPP $\alpha$  and sAPP $\beta$  proteins were detected with anti-APP antibody labeled with MSD SULFO-TAG reagent.

Detection Limits (3 StdDev over bkgnd)	
sAPP $\alpha$	120 pg/mL
sAPP $\beta$	52 pg/mL

# Multiplex A $\beta$ 40, A $\beta$ 42, & sAPP $\beta$ Assay



Recombinant human sAPP $\beta$  was purified from mammalian cells (>95% pure) and synthetic A $\beta$  peptides were combined and diluted in fresh culture medium (DMEM, 10% FBS, Pen/Strep). Samples were added to MSD MULTI-SPOT 4-Spot plates coated with anti-A $\beta$ 40, anti-A $\beta$ 42 and anti-sAPP $\beta$  antibodies on three of the four spatially distinct electrodes per well. The sAPP $\beta$  proteins were detected with anti-APP antibody labeled with MSD SULFO-TAG reagent. The A $\beta$  peptides were detected with biotinylated antibody 4G8 and MSD SULFO-TAG labeled streptavidin.



Concentration	A $\beta$ 40			S/B
	Average	StdDev	%CV	
0	337	41	12	1
0.01 pg/mL	532	519	97	2
0.03 pg/mL	371	81	22	1
0.1 pg/mL	320	30	9	1
0.3 pg/mL	388	91	24	1
1 pg/mL	353	74	21	1
3 pg/mL	329	33	10	1
10 pg/mL	383	34	9	1
30 pg/mL	624	79	13	2
100 pg/mL	1,659	269	16	5
300 pg/mL	6,330	557	9	19
1 ng/mL	19,451	2,158	11	58

A $\beta$ 40 Detection Limit  
(3 StdDev over background)

18 pg/mL

Concentration	A $\beta$ 42			S/B
	Average	StdDev	%CV	
0	25	12	48	1
0.01 pg/mL	48	13	26	2
0.03 pg/mL	41	14	35	2
0.1 pg/mL	48	10	21	2
0.3 pg/mL	28	9	33	1
1 pg/mL	39	9	23	2
3 pg/mL	38	21	56	2
10 pg/mL	45	6	13	2
30 pg/mL	144	18	12	6
100 pg/mL	493	70	14	20
300 pg/mL	2,770	368	13	111
1 ng/mL	11,754	1,305	11	470

A $\beta$ 42 Detection Limit  
(3 StdDev over background)

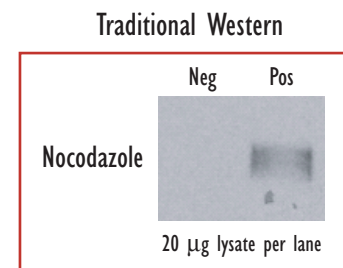
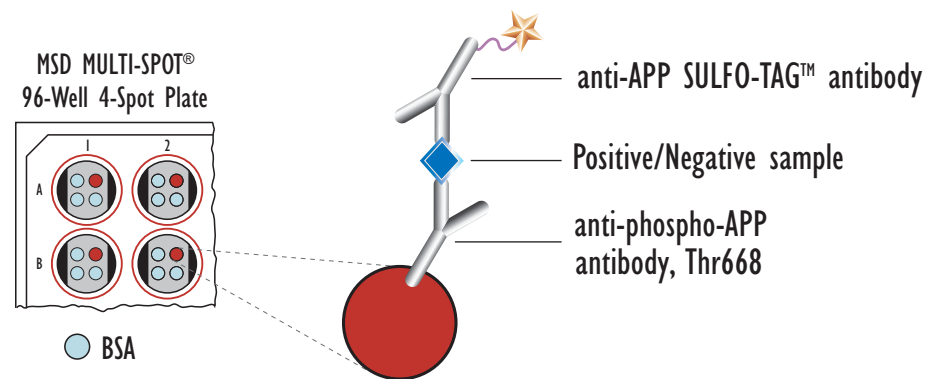
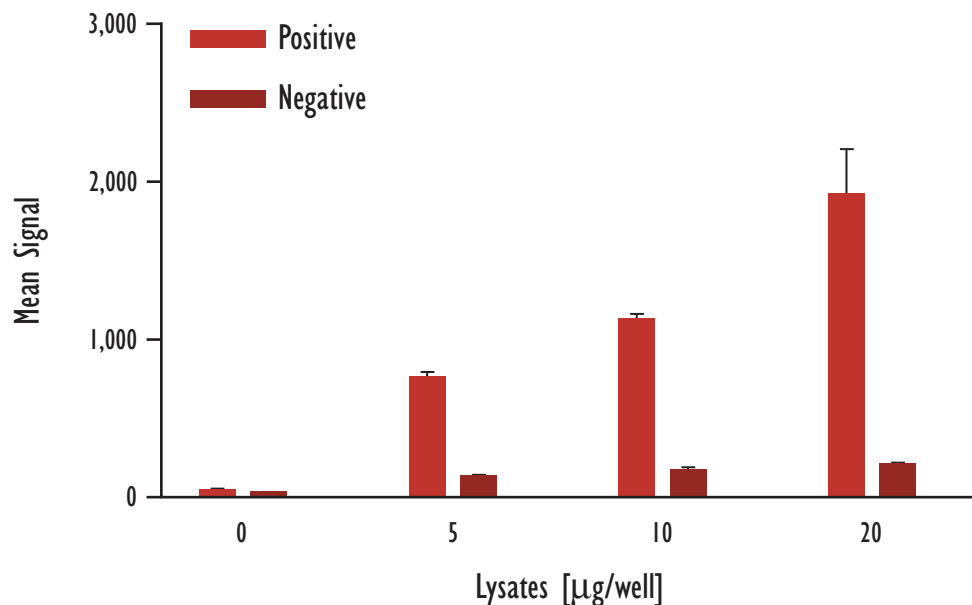
14 pg/mL

Concentration	sAPP $\beta$			S/B
	Average	StdDev	%CV	
0	30	11	39	1
10 pg/mL	40	6	15	1
30 pg/mL	61	7	11	2
100 pg/mL	81	15	19	3
300 pg/mL	106	4	4	4
1 ng/mL	360	19	5	12
3 ng/mL	1,477	112	8	50
10 ng/mL	7,010	940	13	238
30 ng/mL	28,329	2,970	10	960
100 ng/mL	77,963	2,924	4	2,643
300 ng/mL	160,193	16,952	11	5,430
1 $\mu$ g/mL	241,516	20,923	9	8,187

sAPP $\beta$  Detection Limit  
(3 StdDev over background)

140 pg/mL

# Detection of Phosphorylated Alzheimer Precursor Protein (pAPP Thr668)

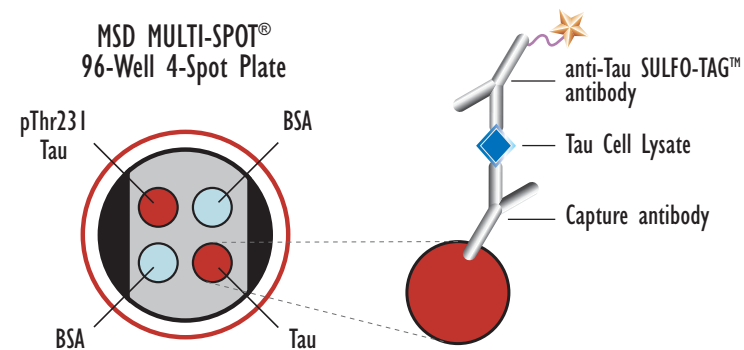
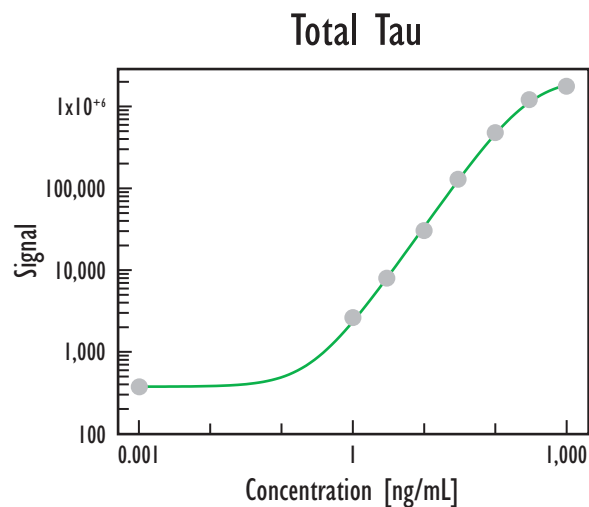
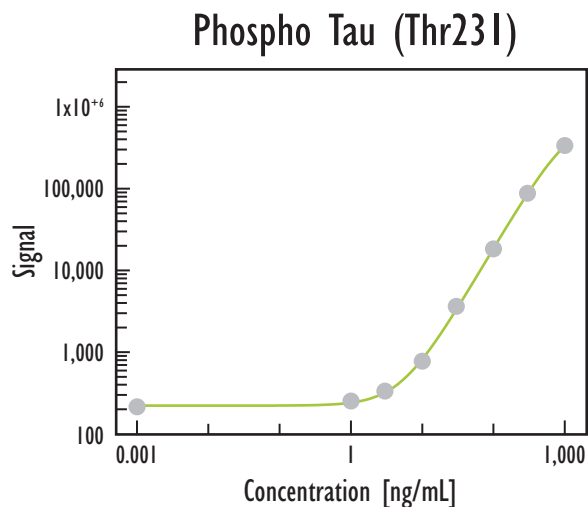


Lysate (µg/well)	Positive			Negative			P/N
	Average	StdDev	%CV	Average	StdDev	%CV	
0	36	12	32	29	2	6	1.3
5	753	42	6	133	7	5	5.6
10	1,122	42	4	168	24	14	6.7
20	1,911	297	16	209	10	5	9.2

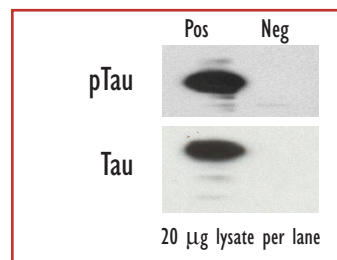
Growing HeLa cells (negative) were treated with nocodazole (1 mg/mL; 20 hr)(positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-phospho-APP antibody on one of the four spatially distinct electrodes per well. Phosphorylated APP was detected with 1 nM anti-APP antibody labeled with MSD SULFO-TAG reagent.



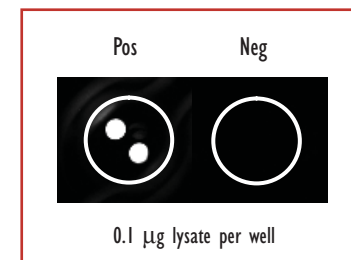
# Detection of Phosphorylated (Thr231) and Total Tau in Human Tau-441 Overexpressing CHO Cells



Traditional Western



MSD Experimental



Extract (ng/well)	Phospho Tau (Thr231)			S/B
	Average	StdDev	%CV	
0	214	14	7	
1	252	27	11	1.2
3	333	8	2	1.6
10	774	75	10	3.6
30	3,621	344	9	16.9
100	18,233	812	4	85.1
300	87,296	5,858	7	407.3
1,000	335,282	26,140	8	1,564.3

Extract (ng/well)	Total Tau			S/B
	Average	StdDev	%CV	
0	369	43	12	
1	2,605	241	9	7.1
3	7,911	29	0	21.4
10	30,304	3,311	11	82.1
30	129,035	16,129	13	349.4
100	481,056	35,082	7	1,302.5
300	1,218,344	62,380	5	3,298.8
1,000	1,778,074	102,560	6	4,814.3

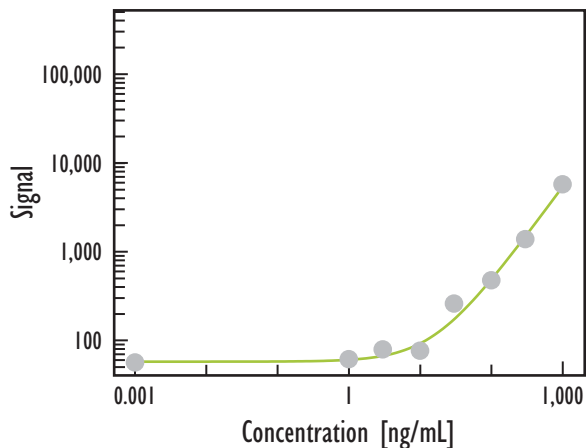
Detection Limit of Lysate [pg/well]	
Phospho Tau (Thr231)	1,480
Total Tau	108

CHO cells were transfected with a human Tau-441 expression plasmid and harvested after 48 hrs. Whole cell lysates were added to MSD MULTI-SPOT 4 Spot 96-well plates coated with anti-total-Tau and anti-phospho-Tau antibodies on two of the four spatially distinct electrodes per well. Phosphorylated and total Tau were detected with anti-total-Tau antibody labeled with MSD SULFO-TAG reagent.

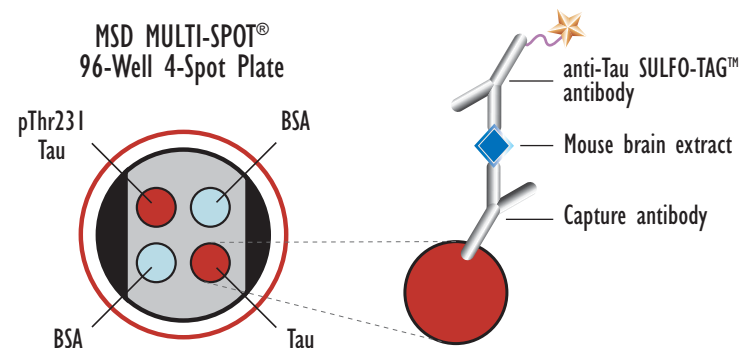
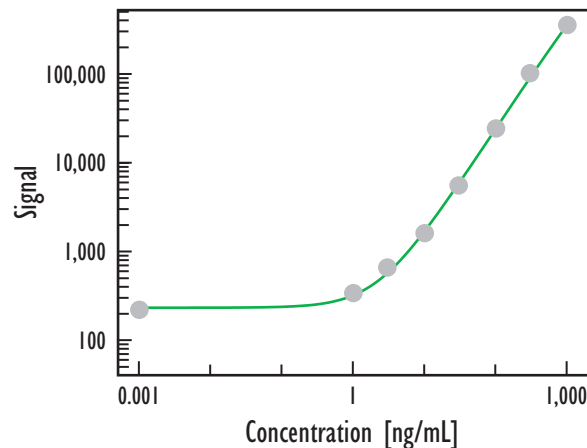


# Detection of Phosphorylated (Thr231) and Total Tau in Mouse Brain Extract

Phospho Tau (Thr231)



Total Tau



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 coated with anti-total-Tau and anti-phospho-Tau antibodies on two of the four spatially distinct electrodes per well. Phosphorylated and total Tau were detected with anti-total Tau antibody labeled with MSD SULFO-TAG reagent.

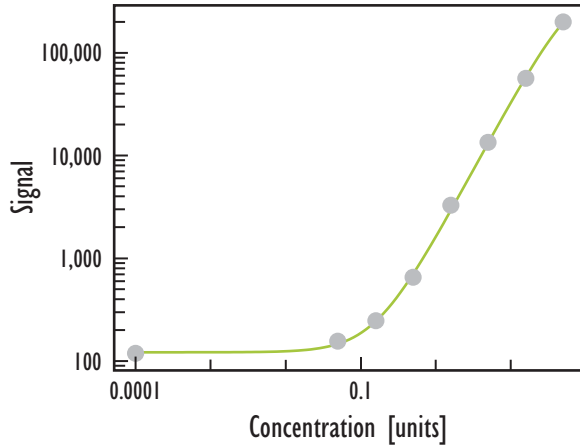
Extract (ng/well)	Phospho Tau (Thr231)			S/B
	Average	StdDev	%CV	
0	56	19	33	
1	61	31	50	1.1
3	79	17	21	1.4
10	76	26	33	1.4
30	259	40	15	4.6
100	475	59	12	8.4
300	1,380	166	12	24.5
1,000	5,726	680	12	101.6

Extract (ng/well)	Total Tau			S/B
	Average	StdDev	%CV	
0	218	37	17	
1	337	16	5	1.5
3	654	45	7	3.0
10	1,597	89	6	7.3
30	5,519	512	9	25.3
100	24,256	1,333	5	111.1
300	102,034	9,042	9	467.3
1,000	356,478	21,275	6	1,632.7

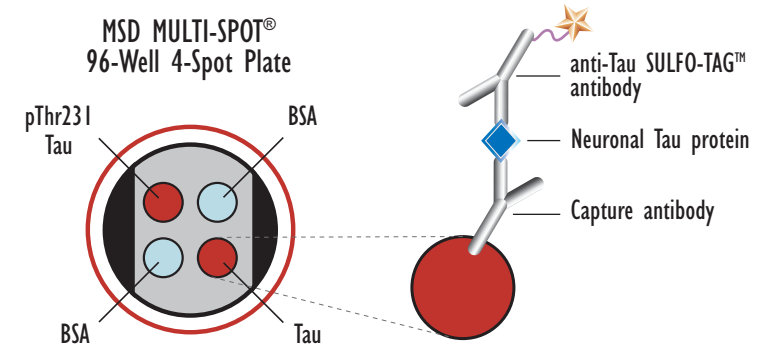
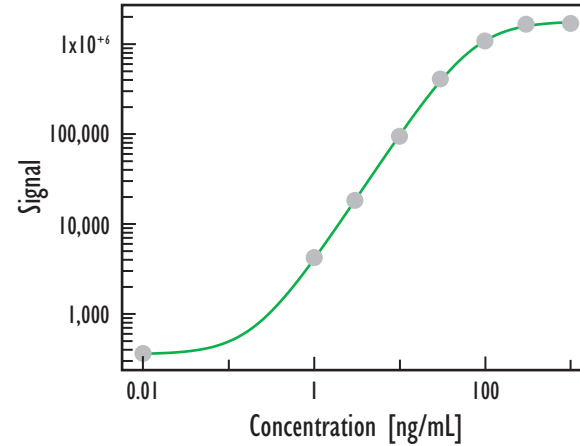
Detection Limit of Mouse Brain Extract [ng/well]	
Phospho Tau (Thr231)	15
Total Tau	1.1

# Quantification of a Neuronal Tau Standard

## Phospho Tau (Thr231)



## Total Tau



Purified neuronal Tau was added to MSD MULTI-SPOT 4 Spot 96-well plates coated with anti-total-Tau and anti-phospho-Tau antibodies on two of the four spatially distinct electrodes per well. Phosphorylated and total Tau were detected with anti-total-Tau antibody labeled with MSD SULFO-TAG reagent.

units pTau(231)	Phospho Tau (Thr231)			S/B
	Average	StdDev	%CV	
0	118	21	17	
0.05	156	22	14	1
0.16	247	10	4	2
0.5	654	48	7	6
1.6	3,272	135	4	28
5	13,396	576	4	113
16	56,554	2,512	4	478
50	199,993	4,688	2	1,690

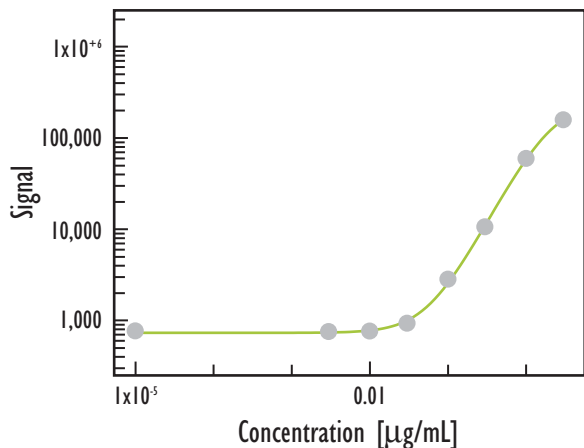
Tau (ng/mL)	Total Tau			S/B
	Average	StdDev	%CV	
0	361	14	4	0
1	4,235	268	6	12
3	18,202	2,139	12	50
10	93,597	23,009	25	259
30	406,815	22,293	5	1,127
100	1,082,204	26,674	2	2,998
300	1,658,455	14,964	1	4,594
1,000	1,686,650	13,417	1	4,672

Detection Limits	
Phospho Tau (Thr231)	0.09 units*
Total Tau	46.1 pg/mL

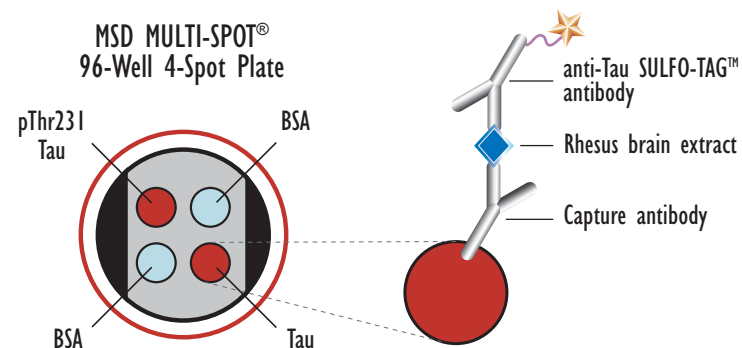
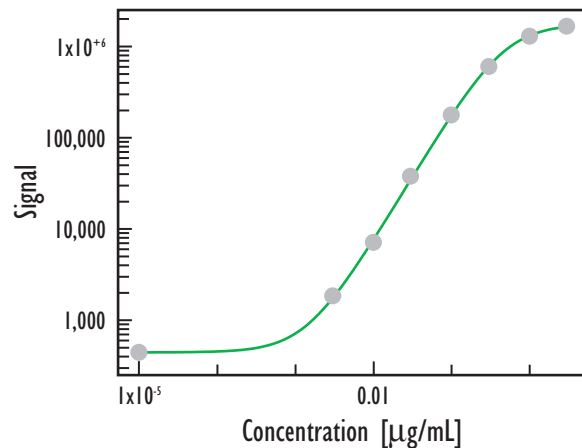
\* Phosphorylated Tau is quantified in units because the percentage of the total Tau that is phosphorylated will vary with different preparations of the standard.

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

Phospho Tau (Thr231)



Total Tau



Extract (µg/well)	Phospho Tau (Thr231)			S/B
	Average	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	2,832	537	19	4
0.3	10,530	1,086	10	14
1	59,382	2,367	4	78
3	157,825	6,848	4	208

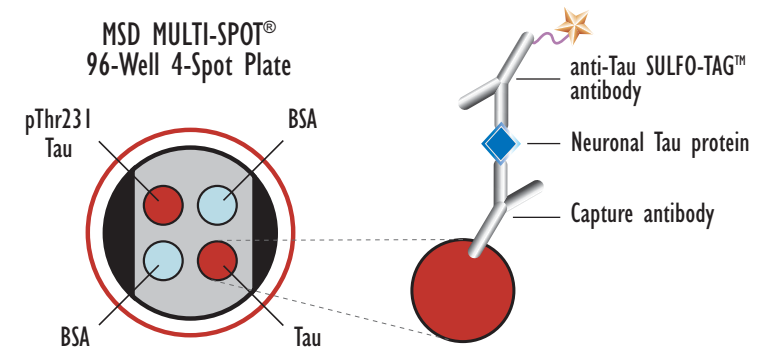
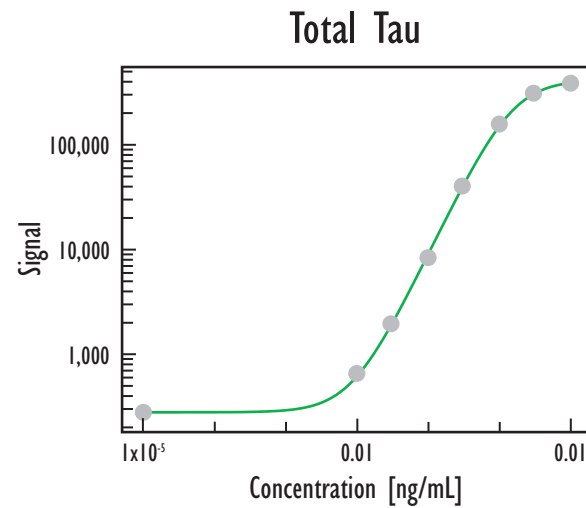
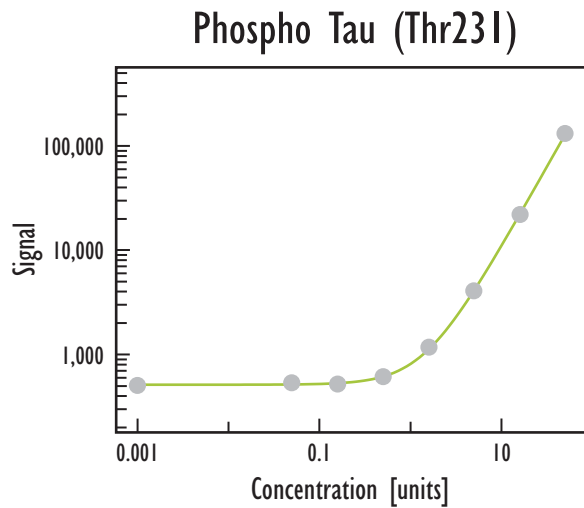
Extract (µg/well)	Total Tau			S/B
	Average	StdDev	%CV	
0	442	42	9	
0.003	1,830	130	7	4
0.01	7,083	531	7	16
0.03	37,746	1,743	5	85
0.1	176,684	4,303	2	400
0.3	600,636	23,678	4	1,358
1	1,296,457	47,217	4	2,932
3	1,668,563	91,566	5	3,773

Detection Limit [ng/well]	
Phospho Tau (Thr231)	19.5
Total Tau	0.6

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 coated with anti-total-Tau and anti-phospho-Tau antibodies on two of the four spatially distinct electrodes per well. Phosphorylated and total Tau were detected with anti-rhesus Tau antibody labeled with MSD SULFO-TAG reagent.



# Rhesus Assay with Neuronal Tau Standard



Purified neuronal Tau was added to MSD MULTI-SPOT 4 Spot 96-well plates coated with anti-total-Tau and anti-phospho-Tau antibodies on two of the four spatially distinct electrodes per well. Phosphorylated and total Tau were detected with anti-rhesus Tau antibody labeled with MSD SULFO-TAG reagent.

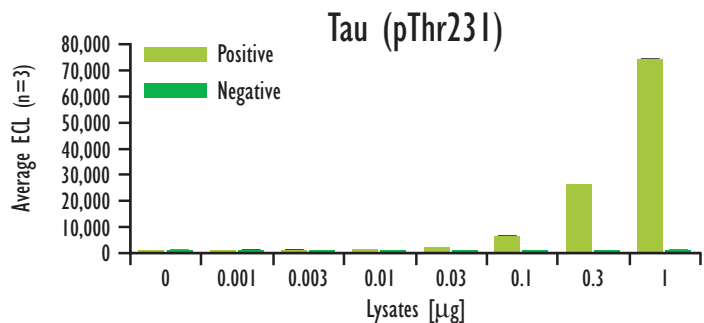
units pTau(231)	Phospho Tau (Thr231)			S/B
	Average	StdDev	%CV	
0	505	12	2	
0.05	531	46	9	1.1
0.16	520	44	8	1.0
0.5	608	39	6	1.2
1.6	1,174	169	14	23
5	4,054	472	12	8.0
16	21,864	1,571	7	43.3
50	130,944	14,086	11	259.1

Tau (ng/well)	Total Tau			S/B
	Average	StdDev	%CV	
0	273	10	3	
1	651	48	7	2.4
3	1,936	556	29	7.1
10	8,355	1,240	15	30.6
30	40,150	4,236	11	147.1
100	158,022	10,024	6	578.8
300	310,437	21,972	7	1,137.1
1,000	380,753	30,747	8	1,394.7

Detection Limits	
Phospho Tau (Thr231)	0.2 units*
Total Tau	156 pg/mL

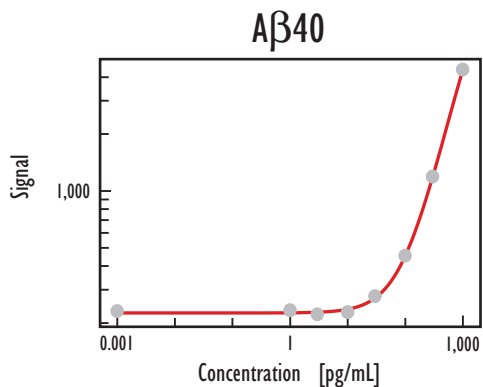
\* Phosphorylated Tau is quantified in units because the percentage of the total Tau that is phosphorylated will vary with different preparations of the standard.

# Multiplex A $\beta$ 40, A $\beta$ 42, sAPP $\alpha$ and Phosphorylated Tau (pThr231) Assay



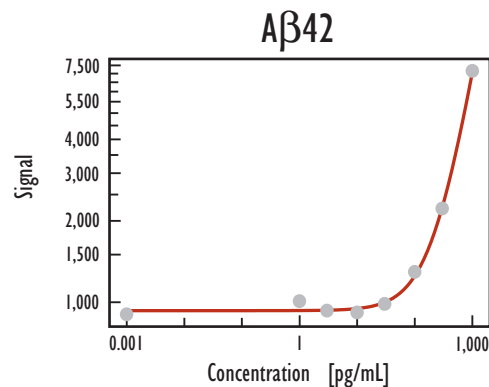
Lysates (µg/well)	pTau (pThr231) Positive			pTau (pThr231) Negative			P/N
	Average	StdDev	%CV	Average	StdDev	%CV	
0	806	303	38	947	27	3	0.9
0.001	962	96	10	984	142	14	1.0
0.003	1,009	94	9	973	83	9	1.0
0.01	1,107	52	5	943	38	4	1.2
0.03	1,868	50	3	982	111	11	1.9
0.1	6,321	252	4	1,000	60	6	6.3
0.3	25,893	136	1	988	43	4	26.2
1	73,907	471	1	820	309	38	90.1

Recombinant human sAPP $\alpha$ , purified from mammalian cells (>95% pure), was diluted in fresh culture medium (DMEM, 10% FBS, Pen/Strep); synthetic A $\beta$  peptides were diluted in MSD Tris Lysis Buffer. Logarithmically growing CHO cells were transfected with a Tau expression plasmid (positive) or mock transfected (no plasmid)(negative), and harvested after 48 hours. All samples were added to MSD MULTI-SPOT<sup>®</sup> 4-Spot plates coated with anti-A $\beta$ 40, anti-A $\beta$ 42, anti-sAPP $\alpha$  and anti-phospho-Tau (pThr231) antibodies on the four spatially distinct electrodes per well. The sAPP $\alpha$  protein and phosphorylated Tau were detected with antibodies labeled with MSD SULFO-TAG<sup>™</sup> reagent. The A $\beta$  peptides were detected with biotinylated antibody 6E10 and MSD SULFO-TAG labeled streptavidin.



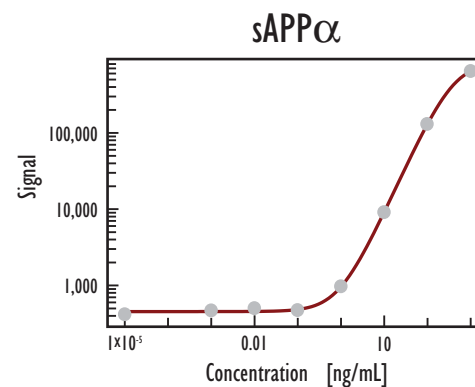
Concentration (pg/mL)	A $\beta$ 40			S/B
	Average	StdDev	%CV	
0	2,311	47	2	
1	2,333	116	5	1.0
3	2,220	38	2	1.0
10	2,279	87	4	1.0
30	2,767	272	10	1.2
100	4,534	274	6	2.0
300	11,898	1,088	9	5.1
1,000	43,821	2,365	5	19.0

A $\beta$ 40 Detection Limit (3 StdDev over zero) **16 pg/mL**



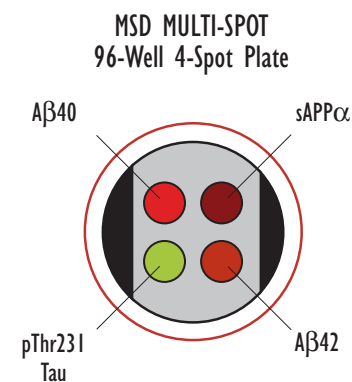
Concentration (pg/mL)	A $\beta$ 42			S/B
	Average	StdDev	%CV	
0	902	40	4	
1	1,008	39	4	1.1
3	930	27	3	1.0
10	917	31	3	1.0
30	985	30	3	1.1
100	1,294	56	4	1.4
300	2,215	67	3	2.5
1,000	7,155	1,234	17	7.9

A $\beta$ 42 Detection Limit (3 StdDev over zero) **37 pg/mL**

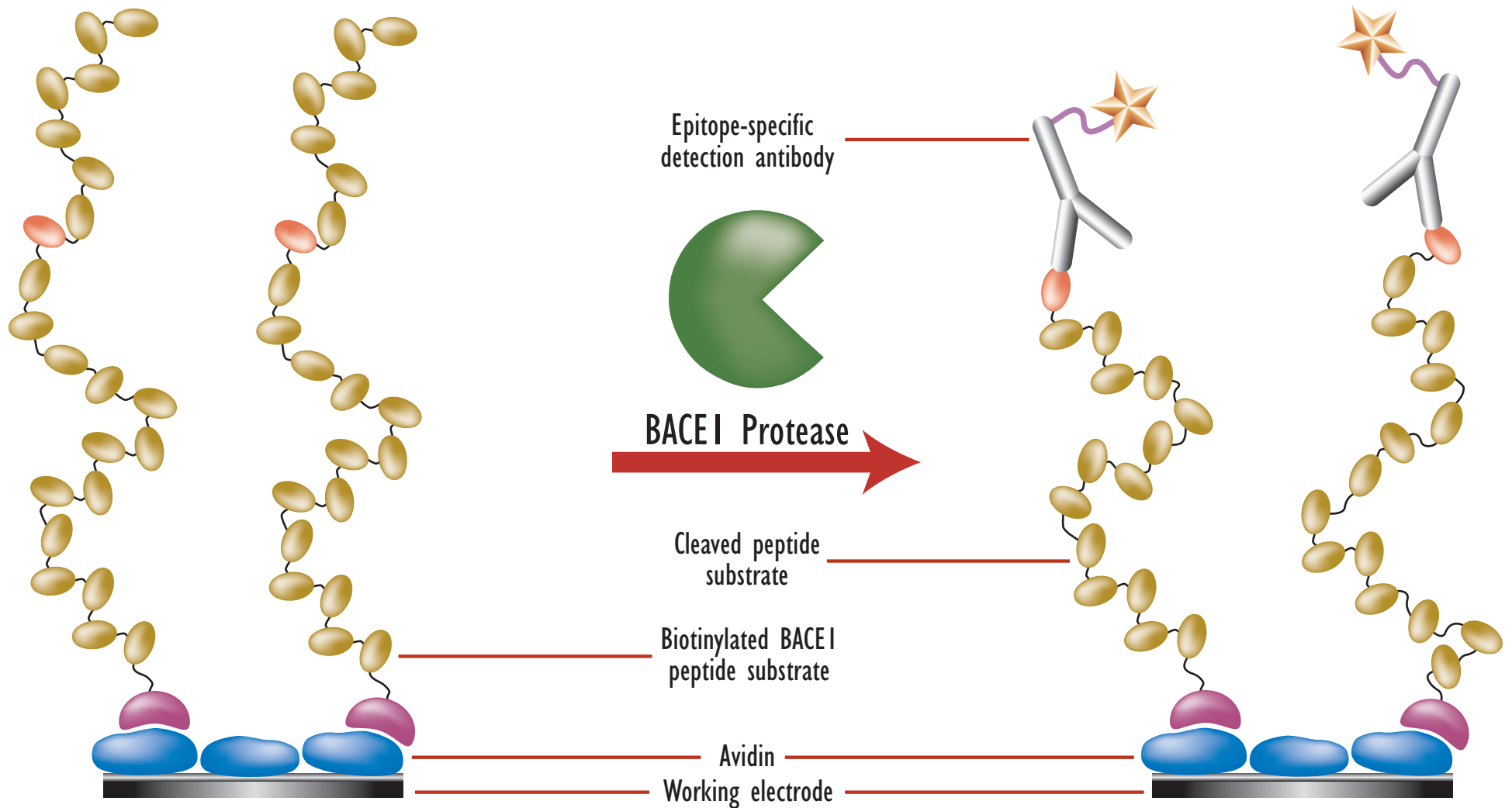


Concentration (ng/mL)	sAPP $\alpha$			S/B
	Average	StdDev	%CV	
0	417	15	4	
0.001	469	9	2	1.1
0.01	503	21	4	1.2
0.1	475	22	5	1.1
1	969	47	5	2.3
10	9,127	591	6	21.9
100	130,668	10,101	8	313.1
1,000	643,830	182,064	28	1,542.7

sAPP $\alpha$  Detection Limit (3 StdDev over zero) **34 pg/mL**

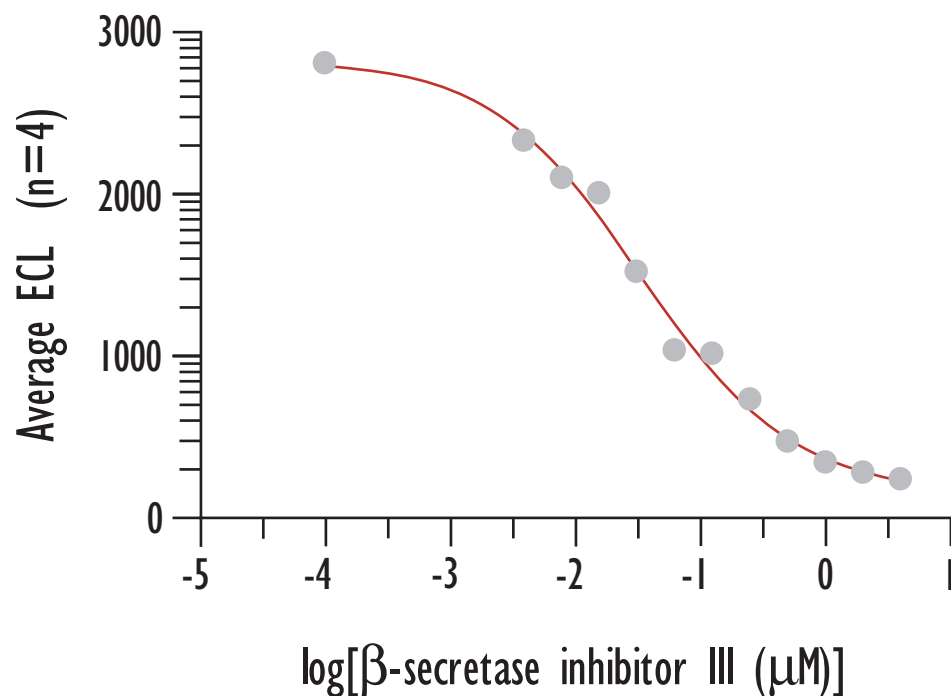


# $\beta$ -secretase Assay: Finding Inhibitors of BACE1



# Inhibition of BACE1 Enzyme with Inhibitor III (GL-189)

## Inhibition of BACE1 Enzyme with Inhibitor III (GL-189)



μM inhibitor	Average	StdDev	CV%	S-B	Activity(%)
0	2822	114	4	2507	100
0.0039	2355	416	18	2040	81
0.0078	1820	79	4	1505	60
0.0156	2031	164	8	1716	68
0.0313	1554	376	24	1239	49
0.0625	1078	241	22	763	30
0.125	1048	270	26	733	29
0.25	764	182	24	449	18
0.5	516	67	13	201	8
1	398	30	8	83	3
2	334	25	8	19	1
4	316	97	31	1	0

Calculated IC<sub>50</sub>=0.034 μM, in good agreement with published value of 0.04 μM (Tung, et al. (2002) J. Med. Chem. 45: 259)

