Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

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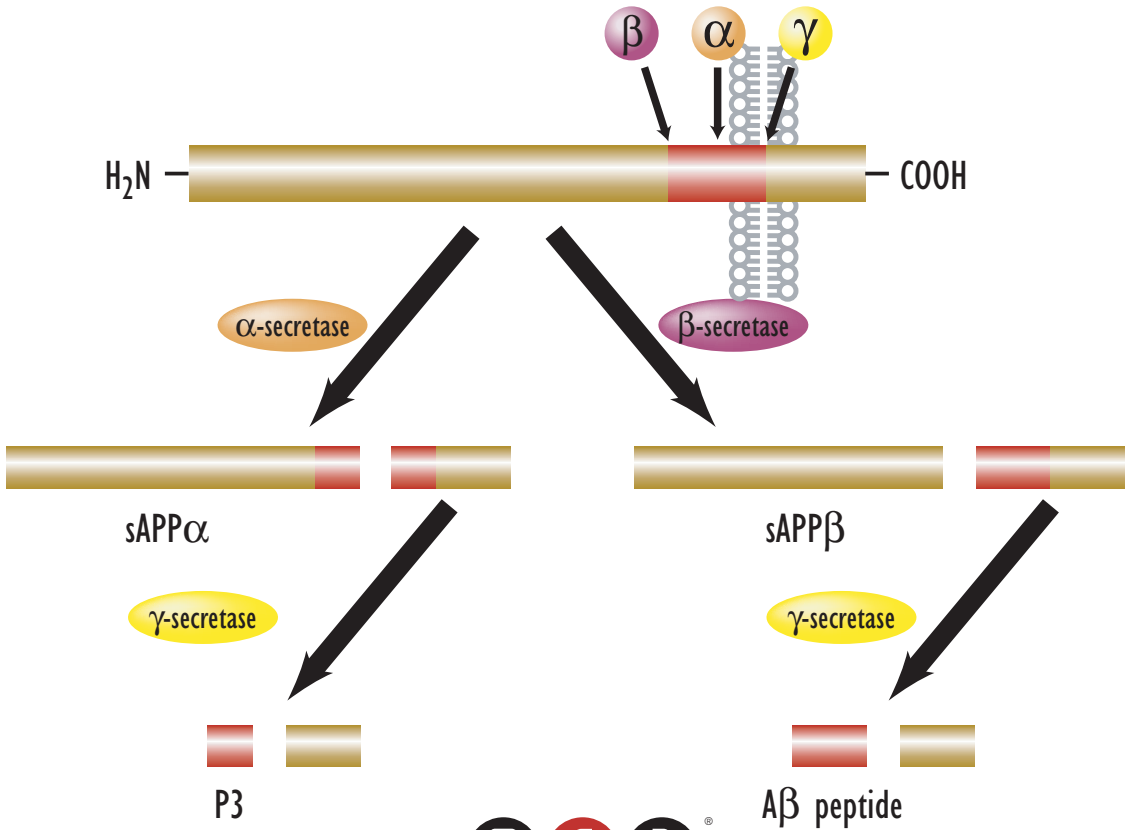
1 Abstract

The Alzheimer precursor protein (APP) is differentially processed into multiple fragments, some of which play a role in Alzheimer's disease (AD). Proteolysis by α-secretase to soluble APPα (sAPPα) precludes the production of Aβ peptides. β- and γ-secretase release sAPPβ and numerous peptides, including Aβ1-40 and Aβ1-42. Aβ1-42 is a major component of amyloid plaques, the protein deposits in the brain that are characteristic of AD. Modulation of APP cleavage may be an effective treatment for AD. We have developed rapid and sensitive multiplex assays to analyze APP products. Aβ1-40 and Aβ1-42 peptides can be quantitated in a single assay well in about 4 hours. Here we show the sensitivity of this assay in multiple sample matrices. Other AD-related assays are also shown, including: a triplex that combines the Aβ peptide assays with sAPPβ; an assay for phosphorylated APP; and, an assay for BACE1 (β-secretase) activity. These assays, along with other AD-related assays and multiplexes that are in development, will provide improved speed and sensitivity from less sample than the currently available assays.

2 Amyloid Precursor Protein (APP) Processing



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Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

3 MSD MULTI-ARRAY™ Technology and MULTI-SPOT® Plates

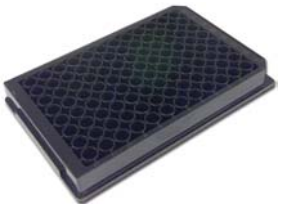
Instrument Features

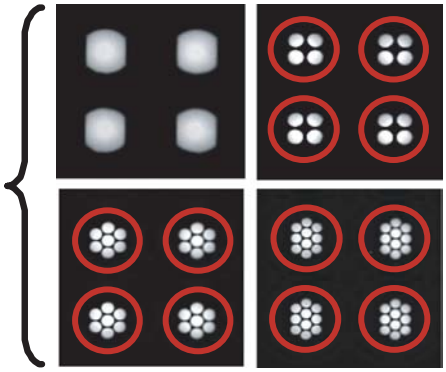
- Highly sensitive imaging detection systems
- Single and multiplex plate formats
- SECTOR Imager 6000 designed for high-throughput screening (HTS)
- Rapid read times
- SECTOR Imager 6000 ideal for assay development
- Electrochemiluminescence (ECL) detection

SECTOR™ Imager 6000

Plate Features

- Disposable plates
- Carbon electrodes with high binding capacity
- Screen printing affords easy patterning
- Suitable electrochemistry for ECL
- A variety of surface treatments, array preparations and coatings are available





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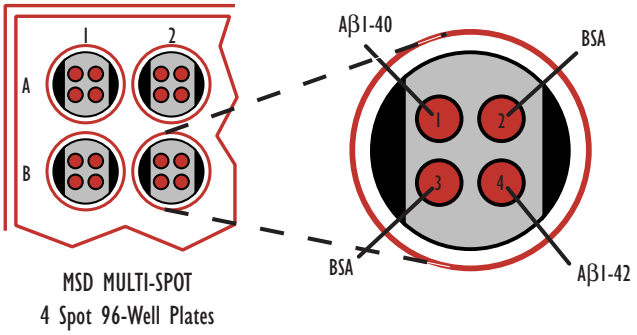
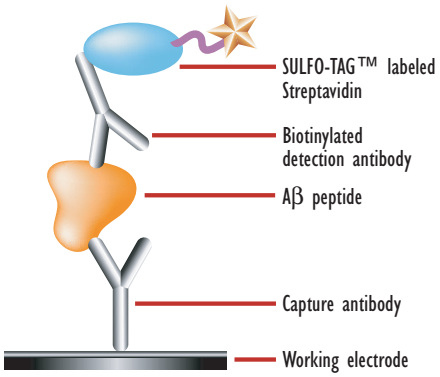
4 Aβ Peptide Duplex Assay Format



MSD MULTI-SPOT
4 Spot 96-Well Plates

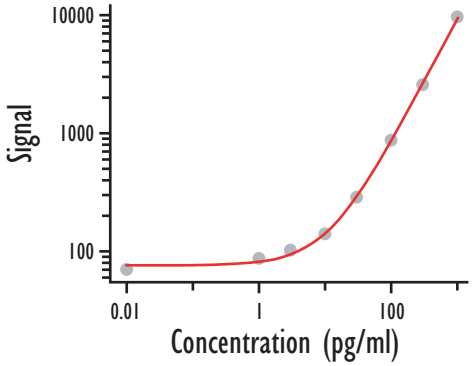


1. MULTI-SPOT 4 Spot 96-Well Plates precoated with capture antibodies are blocked with 150µL of MSD Blocker-A for 1hr and washed with Tris Wash Buffer.
2. 25µL of diluted peptide standards and/or samples are added to the wells and incubated for 1hr with shaking, followed by washing with Tris Wash Buffer.
3. 25µL biotinylated antibodies are added to the wells and incubated for 1 hr with shaking.
4. 25µL MSD SULFO-TAG labeled streptavidin are added to the wells and incubated for 1 hr with shaking, followed by washing with Tris Wash Buffer.
5. 150µL MSD Read Buffer T (with surfactant) are added to the wells and the plate is analyzed on the SECTOR 6000 instrument.

Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

5 Standard Curves in Lysis Buffer

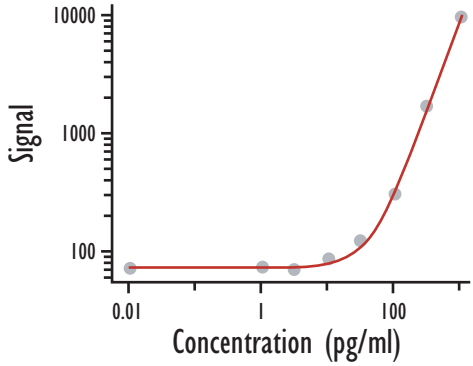
Aβ1-40



pg/ml peptide	Average	S.D.	CV%	S/B
1000	9647	437	5	137.3
300	2577	116	5	36.7
100	873	63	7	12.4
30	287	27	9	4.1
10	141	7	5	2.0
3	102	7	7	1.5
1	87	14	16	1.2
0	70	12	17	

Detection Limit: 4.7 pg/ml

Aβ1-42



pg/ml peptide	Average	S.D.	CV%	S/B
1000	10460	963	9	363.8
300	2769	106	4	96.3
100	812	82	10	28.2
30	246	16	6	8.5
10	97	5	5	3.4
3	53	9	18	1.9
1	38	9	24	1.3
0	29	4	15	

Detection Limit: 24.5 pg/ml

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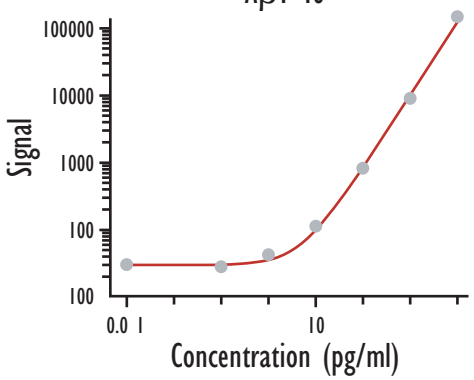
6 Standard Curves in Complete Culture Medium (10% FBS)

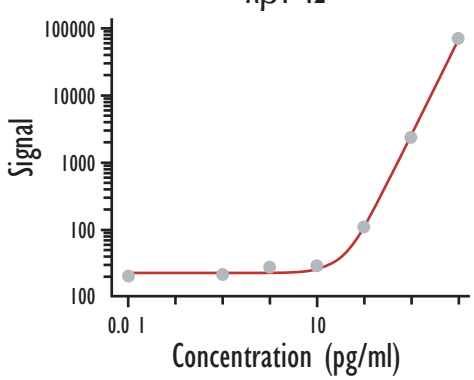
Aβ1-40



pg/ml peptide	Average	S.D.	CV%	S/B
10000	125677	21807	17	3565.3
1000	8741	515	6	248.0
100	769	72	9	21.8
10	112	9	8	3.2
1	48	12	25	1.4
0.1	33	4	13	0.9
0	35	8	24	

Detection Limit: 4.8 pg/ml

Aβ1-42

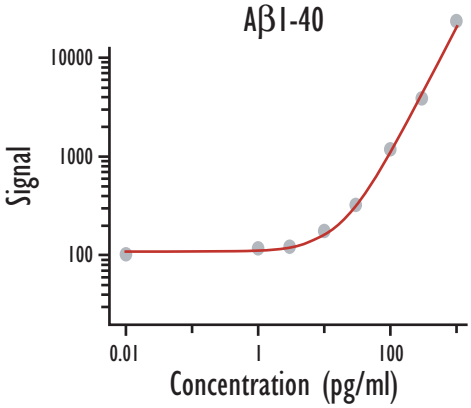


pg/ml peptide	Average	S.D.	CV%	S/B
10000	72149	5130	7	3653.1
1000	2373	86	4	120.1
100	108	14	13	5.4
10	28	7	24	1.4
1	27	2	6	1.4
0.1	21	6	27	1.1
0	19.8	5	23	

Detection Limit: 46.2 pg/ml

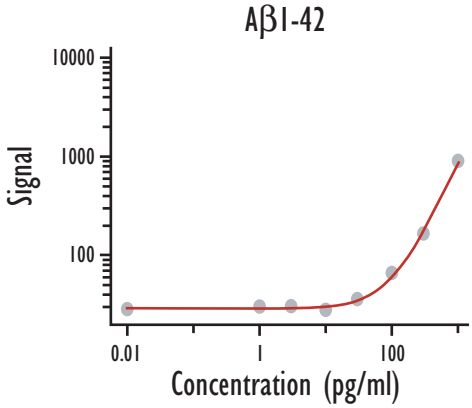
Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

7 Standard Curves in Mouse Serum



pg/ml peptide	Average	S.D.	CV%	S/B
1000	23428	3636	16	164.6
300	3865	608	16	27.2
100	1179	67	6	8.3
30	323	26	8	2.3
10	176	14	8	1.2
3	122	13	10	0.9
1	117	4	3	0.8
0	102	7	7	

Detection Limit: 5.2 pg/ml



pg/ml peptide	Average	S.D.	CV%	S/B
1000	908	164	18	18.7
300	167	8	5	3.4
100	67	11	16	1.4
30	36	4	12	0.7
10	28	6	19	0.6
3	31	5	15	0.6
1	31	4	13	0.6
0	29	8	28	

Detection Limit: 94.7 pg/ml

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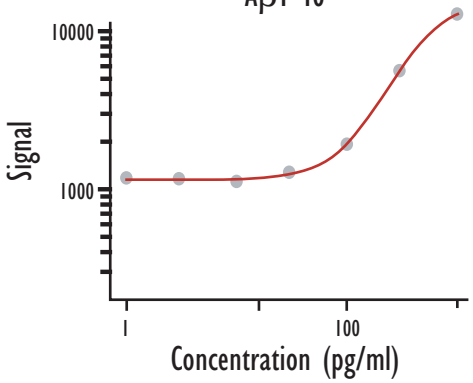
8 Standard Curves In Human Cerebrospinal Fluid (CSF)

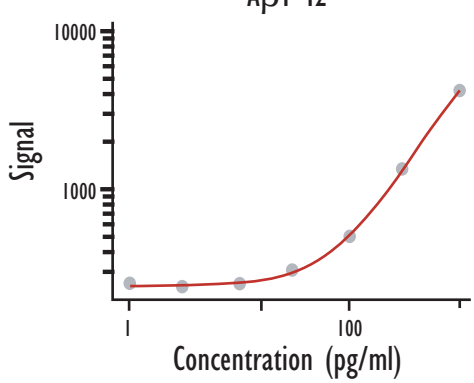
Aβ1-40



pg/ml peptide	Average	S.D.	CV%	S/B
1000	12816	287	2	10.9
300	5619	24	0	4.8
100	1925	177	9	1.6
30	1278	80	6	1.1
10	1118	110	10	0.9
3	1163	59	5	1.0
1	1177	47	4	

Detection Limit: 42.0 pg/ml

Aβ1-42



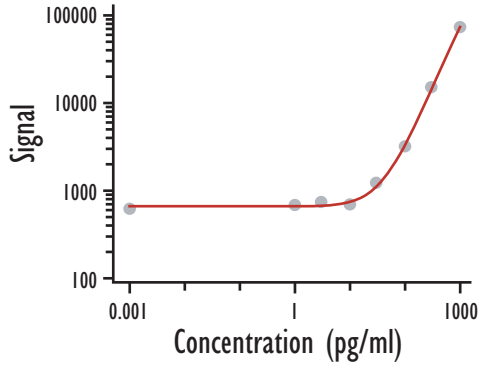
pg/ml peptide	Average	S.D.	CV%	S/B
1000	4202	97	2	16.6
300	1341	67	5	5.3
100	503	25	5	2.0
30	308	24	8	1.2
10	252	16	6	1.0
3	242	8	3	1.0
1	254	16	6	

Detection Limit: 31.5 pg/ml

Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

9 Standard Curves in Human Serum

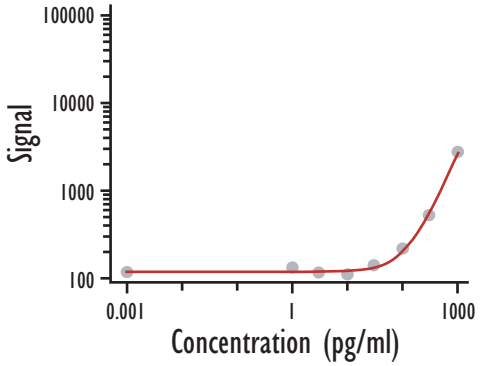
Aβ1-40



pg/ml peptide	Average	S.D.	CV%	S/B
1000	73403	12793	17	118.4
300	15137	1637	11	24.4
100	3195	455	14	5.2
30	1228	102	8	2.0
10	695	269	39	1.1
3	742	105	14	1.2
1	685	70	10	1.1
0	620	57	9	

Detection Limit: 13.0 pg/ml

Aβ1-42

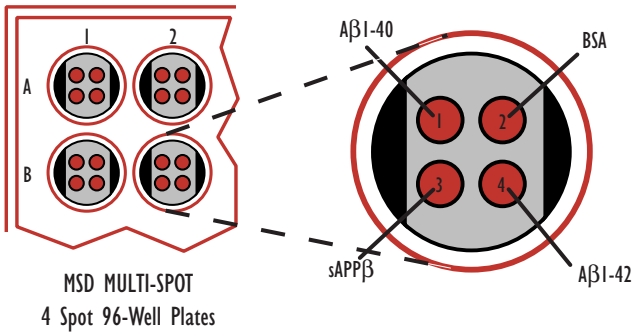


pg/ml peptide	Average	S.D.	CV%	S/B
1000	2781	443	16	23.7
300	526	37	7	4.5
100	218	26	12	1.9
30	141	14	10	1.2
10	111	29	26	0.9
3	116	12	10	1.0
1	133	21	16	1.1
0	118	14	12	

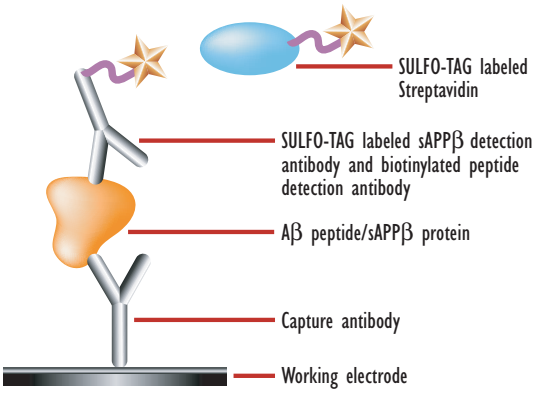
Detection Limit: 59.0 pg/ml

Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

10 Aβ peptides and sAPPβ Triplex Assay Format

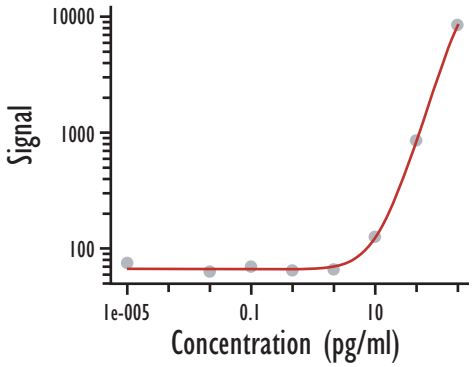


MSD MULTI-SPOT
4 Spot 96-Well Plates



11 Aβ Peptide / sAPPβ Triplex – Standards Diluted in Culture Medium

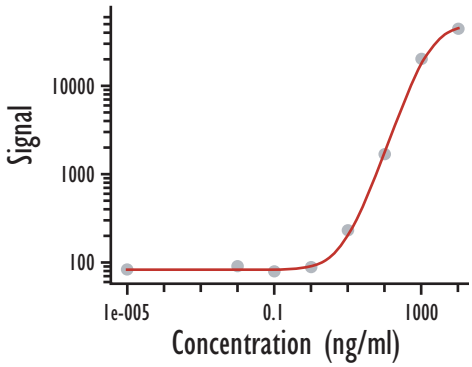
Aβ1-40



pg/ml	Average	S.D.	CV%
1000	8477	1439	17
100	853	102	12
10	126	12	10
1	66	13	20
0.1	65	9	13
0.01	70	20	29
0.001	63	7	11
0	75	12	16

Detection Limit: 8.2 pg/ml

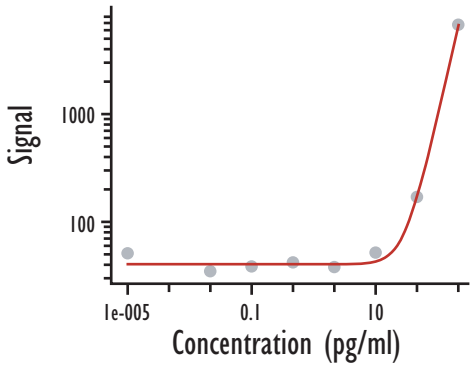
sAPPβ



ng/ml	Average	S.D.	CV%
10000	44163	2663	6
1000	20159	948	5
100	1678	435	26
10	231	9	4
1	88	9	10
0.1	79	10	13
0.01	91	11	12
0	83	3	4

Detection Limit: 1.2 ng/ml

Aβ1-42



pg/ml	Average	S.D.	CV%
1000	6732	1587	24
100	170	13	8
10	52	11	21
1	38	6	17
0.1	42	9	22
0.01	39	15	40
0.001	35	5	15
0	51	12	23

Detection Limit: 53.5 pg/ml

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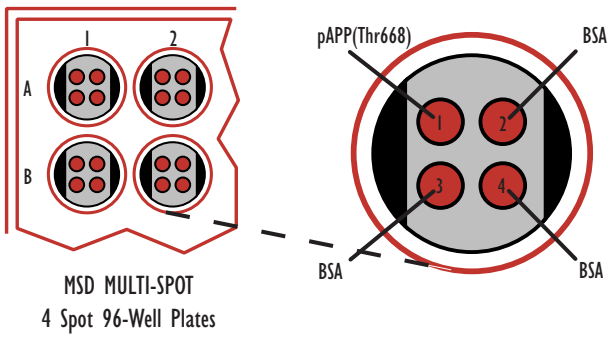
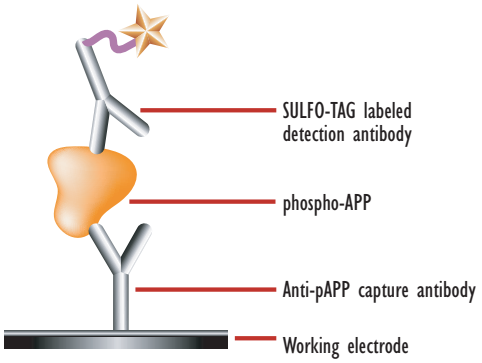
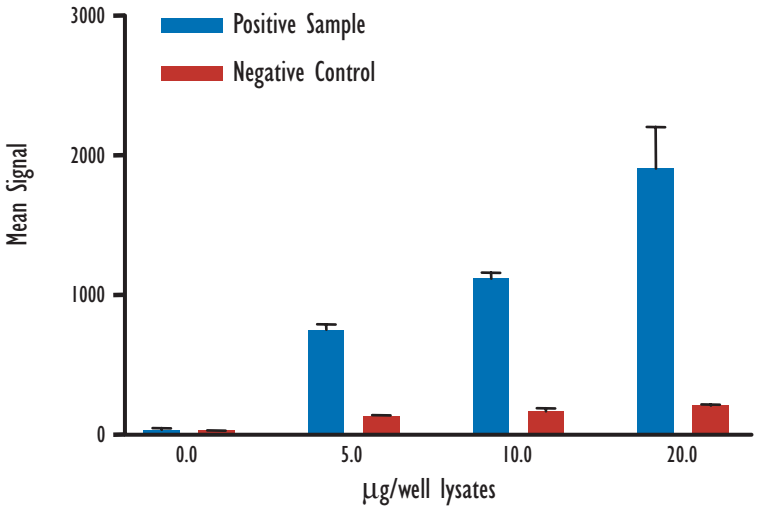
12 Phosphorylated APP (Thr668) Assay Format



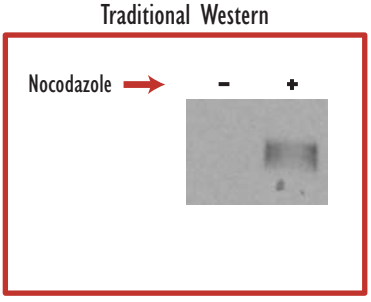
MSD MULTI-SPOT
4 Spot 96-Well Plates



13 pAPP in HeLa Cells



µg/well Lysate	Positive			Negative			S/B
	Ave	Std.Dev.	%CV	Ave	Std.Dev.	%CV	
0	36	12	32	29	2	6	1.3
5	753	42	6	133	7	5	5.6
10	1122	42	4	168	24	14	6.7
20	1911	297	16	209	10	5	9.2



Cell lysates were prepared from HeLa cells treated with 1µg/mL nocodazole for 20hr or control untreated cells. 20µg lysate was loaded per lane.

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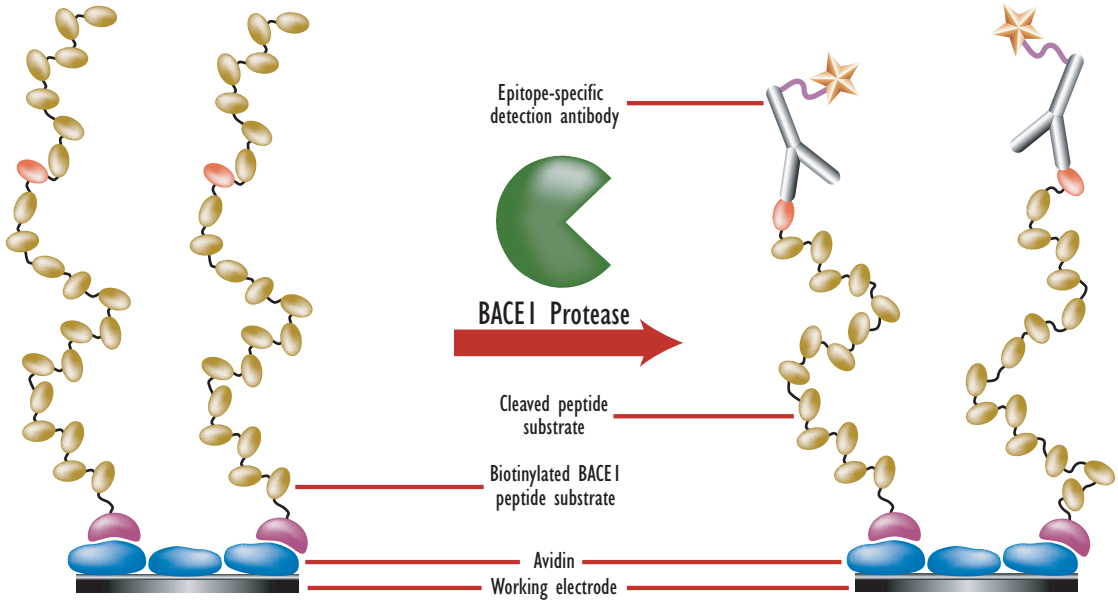
14 A β-secretase assay: Finding Inhibitors of BACE1



1. MULTI-SPOT 4 Spot 96-Well Plates precoated with avidin are incubated with Biotinylated BACE1 peptide substrate for 30 min and then washed.
2. BACE1 enzyme and/or other samples are added to the wells and incubated for 1 hr, followed by washing.
3. MSD SULFO-TAG detection antibody is added to the wells and incubated for 30 min, followed by washing.
4. 150µL MSD Read Buffer T (with surfactant) are added to the wells and the plate is analyzed on the SECTOR 6000 instrument.

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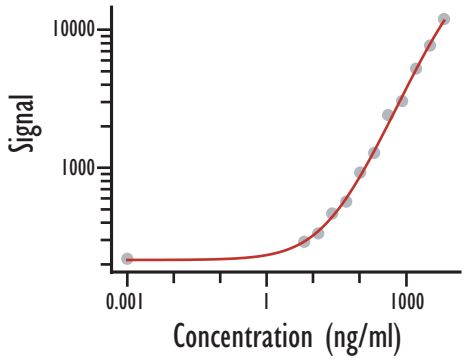
14 Aβ-secretase assay: Finding Inhibitors of BACE1 (continued)

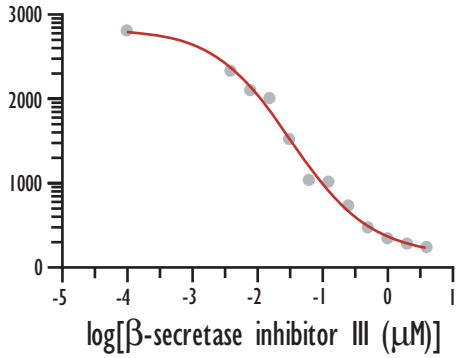
Titration of BACE1 Enzyme



ng/ml enzyme	Average	S.D.	CV%	S/B
6670	11940	1709	14	54.6
3335	7681	519	7	35.1
1667	5223	768	15	23.9
834	3040	347	11	13.9
417	2406	122	5	11.0
208	1281	185	14	5.9
104	923	115	12	4.2
52	568	109	19	2.6
26	466	14	3	2.1
13	334	15	5	1.5
6.5	290	37	13	1.3
0	219	28	13	

Detection Limit: 7.6 ng/ml

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



μM inhibitor	Average	S.D.	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 IC50=0.034 μM, in good agreement with published value of 0.04 μM (Ermolieff et al.).

Multiplex Detection of Alzheimer's Disease-related Proteins and Peptides

15 Conclusions

- We have developed highly specific multiplexed assays for simultaneously measuring the Aβ1-40 and Aβ1-42 peptides, and a specific assay for sAPPβ, which can be measured in a multiplex with the Aβ peptides.
- A cell-based assay has been developed for the detection of phosphorylated APP in cell lysates.
- A very rapid and simple *in vitro* assay for BACE1 activity has been demonstrated, and is being further developed as a cell-based assay.
- Multiple proteolytic products of APP can be assayed simultaneously in a single well by using specific antibodies immobilized on MSD MULTI-SPOT plates. The MULTI-ARRAY technology-based assay can be readily adapted to any protein for which antibodies are available.
- The assays are specific and afford higher throughput replacements to gold-standard methods like ELISAs. Assaying multiple species in the same well reduces the labor involved and the amount of sample required.
- The assays can be easily automated, and are suitable for HTS.

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