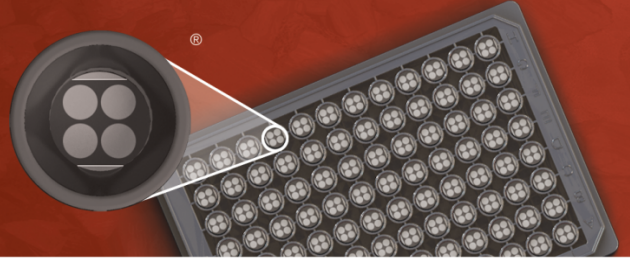
MSD® Neurodegeneration Assays

Amyloid Beta (Aβ) Peptides and Tau



Neurodegeneration
BioProcess
Cardiac
Cell Signaling
Clinical Immunology
Cytokines
Growth Factors
Hypoxia
Immunogenicity
Inflammation
Metabolic
Oncology
Toxicology
Vascular

Ordering Information

MSD Customer Service
Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com
www.mesoscale.com/support

Scientific Support

Phone: 1-240-314-2798
Email: ScientificSupport@mesoscale.com
www.mesoscale.com/support

Company Address

MESO SCALE DISCOVERY®
A division of
Meso Scale Diagnostics, LLC.
1601 Research Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com®

For Research Use Only. Not for use in diagnostic procedures.

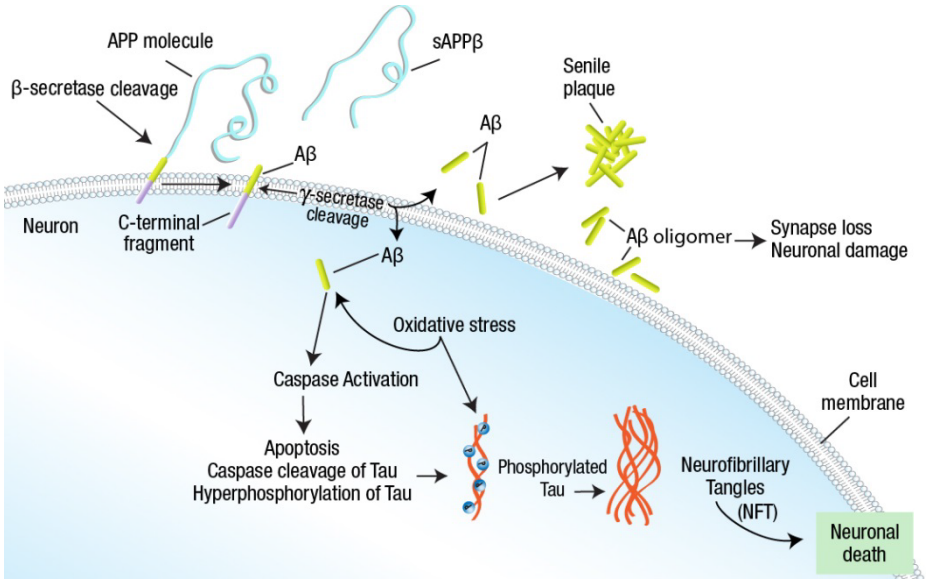


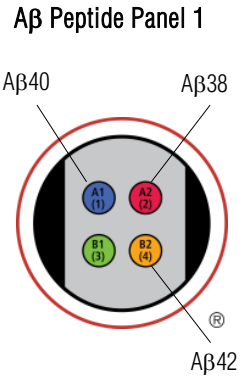
Figure 1. Amyloid precursor protein processing and putative neurodegeneration pathway in Alzheimer's disease.[1]

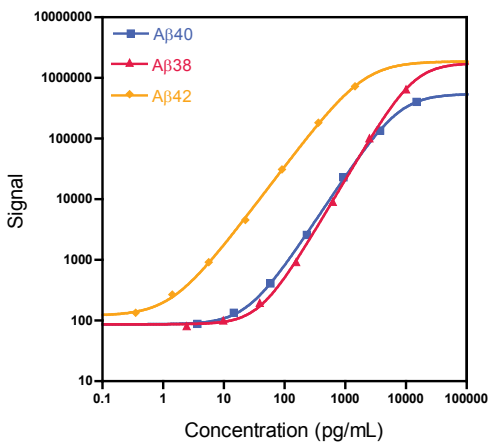
Validated assays for high quality and reproducible results

- ✓ Validated
- ✓ Reproducible
- ✓ Sensitive
- ✓ Fast

Aβ Peptide Panel 1 (6E10)			
Analyte	Median LLOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
Aβ40	9.97	25.0	7000
Aβ38	16.7	60.0	8475
Aβ42	0.368	3.13	1271

Numerous studies over the past decade affirm the utility of measuring Aβ peptides and tau protein biomarkers in cerebrospinal fluid (CSF) for the prognosis of Alzheimer's disease (AD).[2,3] In order to reliably measure these markers in biological samples, there is a need for sensitive, precise immunoassays that provide consistent measurements across manufacturing runs and users.[4] MSD has developed fully validated research-use only Aβ peptide and tau assays under rigorous design control and according to "Fit for Purpose" principles[5] to provide the analytical performance needed for the reliable measurement of these biomarkers in human CSF. The table above indicates the superior sensitivity of the assays included in the Aβ Peptide Panel 1 (6E10) Kit. Typical standard curves from the panel are illustrated below. Visit www.mesoscale.com/assays for a complete list of available Alzheimer's disease products.





Spot the Difference®

Reliable Performance: Control Testing

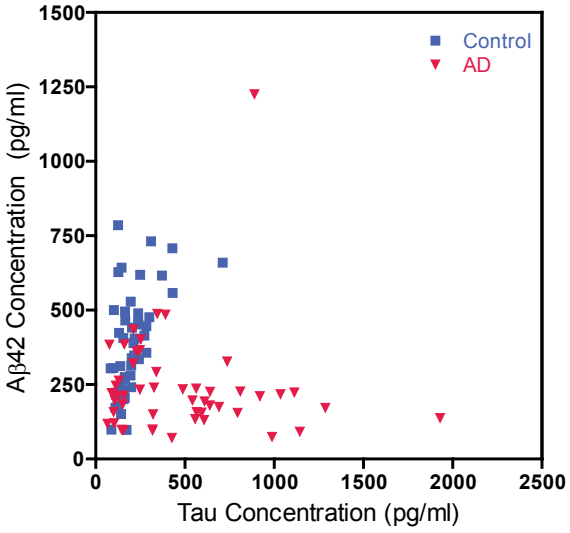
An integral part of assay validation is evaluation of performance across multiple kit lots, which involves characterization of assay precision across multiple runs and analysts within and across each lot. Precision data for controls is shown below. The data is representative of 38 runs and 5 analysts across 3 kit lots. While the typical specification for precision is a concentration CV of less than 25%, for the assays in this panel, the CVs obtained were well below 10%, which is reflective of the high quality of the assays.

Analyte	Control ID	Ave. Conc. (pg/mL)	Ave. Intra-plate %CV	Ave. Inter-plate Conc. %CV	Inter-Kit Lot Conc. %CV
Aβ40	CSF Control 1	9415	4.4	6.0	5.5
	CSF Control 2	3111	4.2	6.9	6.1
	CSF Control 3	1933	4.9	8.3	4.2
Aβ38	CSF Control 1	5276	3.1	6.1	5.3
	CSF Control 2	1471	3.6	7.2	0.6
	CSF Control 3	705	4.0	7.8	3.0
Aβ42	CSF Control 1	1925	3.0	5.1	9.2
	CSF Control 2	513	2.4	5.4	9.0
	CSF Control 3	263	2.7	7.0	8.0

Results shown are from the MSD Aβ Peptide Panel 1 (6E10) Kit

The Use of Biomarkers in Clinical Trials

Biomarkers associated with neurodegenerative diseases are emerging as critical tools for powering clinical trials in mildly symptomatic or asymptomatic individuals. The use of biomarkers or ratios of biomarkers as inclusion/exclusion criteria is projected to identify the individuals most likely to benefit from a candidate therapeutic and to reduce trial costs. Successful implementation of biomarkers in international clinical trials will require stable assay cut-points that discriminate among individuals and correlate with independent diagnoses or outcomes. The validated assays from MSD have been used to establish cut-points that discriminate healthy controls from mild cognitive impairment (MCI) and Alzheimer's disease (AD) patients.[6] Cut-point values may depend on several analytical factors. Therefore, we recommend that each laboratory establish their own cut-point values.



Samples	CSF Tau (pg/mL)	CSF Aβ42 (pg/mL)
Control Mean ± SEM (N)	195 ± 15.8 (44)	404 ± 26.1 (42)
MCI Mean ± SEM (N)	433 ± 44.5* (46)	303 ± 25.7* (45)
AD Mean ± SEM (N)	484 ± 59.3* (45)	238 ± 25.31* (49)
Cut-point	> 319	< 240

*p<0.05, AD or MCI vs. control

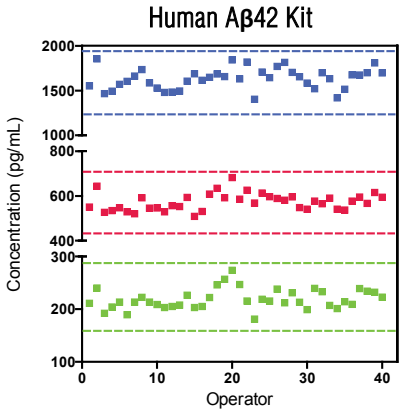
SEM = Standard error of the mean

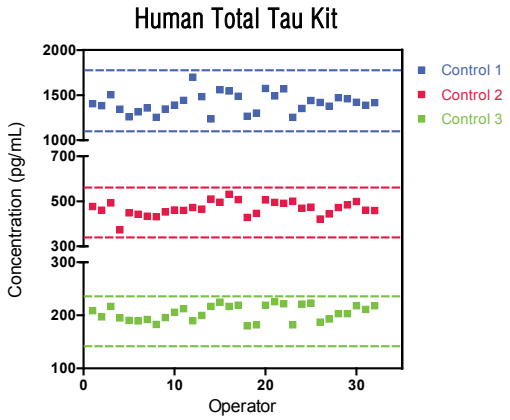
MSD Neurodegeneration Assays

Reproducibility Across Multiple Sites

In our commitment to providing state-of-the-art tools for biomarker measurements and superior customer support, MSD has implemented a training program which is available to the AD research community, including academic institutions, biopharmaceutical institutions, and contract research organizations (CROs). The program aim is to assist researchers in achieving accurate and precise measurements across multiple sites. As part of the program, a highly skilled Field Application Scientist provides training on best practices for conducting MSD's neurodegeneration assays. The analyst then successfully conducts multiple assay runs, generating calibration curves, measuring controls, and conducting sample dilution linearity, all within set specifications.

Representative results from multi-center training using control samples from the MSD Neurodegeneration Control Pack 1 (6E10) (catalog # C41LB-1) are shown below. Analysts at 19 international sites were trained on MSD's Human Aβ42 and Human Total Tau products. Data represents at least 165 replicates for each control. The horizontal dotted lines indicate ±25% recovery of each control relative to the nominal value established at MSD for each control. The excellent inter-analyst CVs for all controls are provided in the table below.

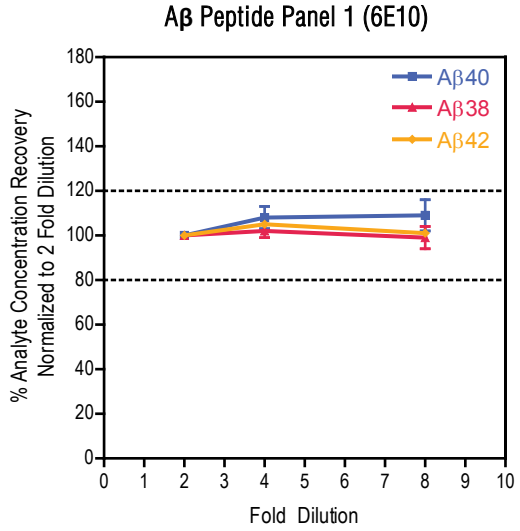




Analyte	Control ID	Inter-analyst %CV
Aβ42	Control 1	7.1
	Control 2	6.6
	Control 3	8.7
Tau	Control 1	7.8
	Control 2	6.8
	Control 3	7.2

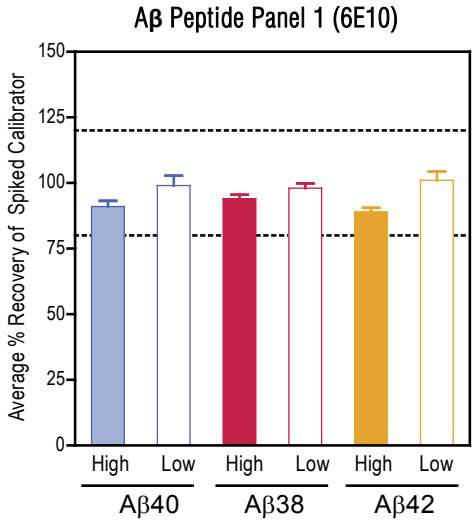
Overcoming Matrix Challenges

Interference from CSF matrix presents a challenge when measuring neurodegeneration biomarkers. The dilution linearity profile shown below exemplifies the excellent matrix tolerance of MSD neurodegeneration assays in CSF. Ten human CSF samples were evaluated using the Aβ Peptide Panel 1 (6E10) Kit. The average recovery relative to the measured concentration at 2-fold dilution is plotted as a function of sample dilution factor.



Spike Recovery

Recovery of Aβ peptides spiked at high and low levels into human CSF was evaluated as part of assay validation. All assays in the Aβ Peptide Panel 1 (6E10) Kit gave excellent spike recovery that was within 80% to 100% of the expected values at both levels. Representative results from 10 human CSF samples are shown below.



MSD Neurodegeneration Assays

MSD Validated Assays for Biomarkers of Alzheimer's Disease

MSD's validated neurodegeneration assays are offered in V-PLEX® and V-PLEX Plus configurations. V-PLEX kits include individual calibrators, detection antibodies, diluents, reagents, product inserts, and certificates of analysis. V-PLEX Plus kits contain controls, wash buffer, and plate seals in addition to V-PLEX kit components. All V-PLEX and V-PLEX Plus kits ship in 1 to 3 days.

Kit Name	Analyte	V-PLEX Kits Catalog Numbers	V-PLEX Plus Kits Catalog Numbers
		1/-5/-25 plates	1/-5/-25 plates
Multiplex Kits			
Aβ Peptide Panel 1 (6E10)	Aβ38, Aβ40, Aβ42	K15200E-1/-2/-5	K15200G-1/-2/-5
Aβ Peptide Panel 1 (4G8)	Aβ38, Aβ40, Aβ42	K15199E-1/-2/-5	K15199G-1/-2/-5
Single Assay Kits			
Aβ40 Peptide (6E10)	Aβ40	K150SKE-1/-2/-5	K150SKG-1/-2/-5
Aβ38 Peptide (6E10)	Aβ38	K150SIE-1/-2/-5	K150SIG-1/-2/-5
Human Aβ42 (6E10)	Aβ42	K151LBE-1/-2/-5	K151LBG-1/-2/-5
Aβ40 Peptide (4G8)	Aβ40	K150SJE-1/-2/-5	K150SJG-1/-2/-5
Aβ38 Peptide (4G8)	Aβ38	K150SHE-1/-2/-5	K150SHG-1/-2/-5
Aβ42 Peptide (4G8)	Aβ42	K150SLE-1/-2/-5	K150SLG-1/-2/-5
Human Total Tau	Tau	K151LAE-1/-2/-5	K151LAG-1/-2/-5

Supplementary Materials		
Item	**Description**	**Catalog Number**
Neurodegeneration Control Pack 1 (6E10)	Multi-analyte controls in diluent that mimics CSF	C41LB-1
Neurodegeneration Control Pack 1 (4GB)		C40RQ-1
Aβ1-38 Peptide (6E10)	Synthetic peptide calibrators in diluent that mimics human CSF	C00NZ-2
Aβ1-40 Peptide (6E10)		C00OA-2
Aβ1-42 Peptide (6E10)		C01LB-2
Aβ1-38 Peptide (4G8)		C002Y-2
Aβ1-40 Peptide (4G8)		C002Z-2
Aβ1-42 Peptide (4G8)		C003A-2
Aβ 4G8 Antibody (1 plate)	SULFO-TAG™ conjugated antibody specific for human and rodent peptides	D20RQ-2
Aβ 4G8 Antibody (5 Plate)		D20RQ-3
Human Aβ 6E10 Antibody (1 plate)	SULFO-TAG conjugated antibody specific for human peptides	D21LB-2
Human Aβ 6E10 Antibody (5 Plate)		D21LB-3

Other MSD Neurodegeneration Assays

In addition to the validated assays listed above, MSD offers a suite of standard biomarker assays for the measurement of neurodegeneration biomarkers. Some of these assays are listed below. Visit www.mesoscale.com/assays for a complete list of available products.

- ❑ Soluble APPα (human)
- ❑ Soluble APPβ (human, rodent)
- ❑ Soluble APPβ Swedish (human, rodent)
- ❑ Soluble APPα/β duplex (human)
- ❑ Phospho-Tau (Thr231) (human, mouse)
- ❑ Phospho-Tau (Ser262) (mouse)
- ❑ Phospho-Tau (Ser396) (human)
- ❑ Phospho-Tau (Thr181) (human)
- ❑ Phospho-Tau (Thr231)/Total Tau duplex (human, mouse)

References

1. Rohn TT. The triggering receptor expressed on myeloid cells 2: "TREM-ming" the inflammatory component associated with Alzheimer's disease. Oxid Med Cell Longev. 2013;2013:860959.
2. Blennow K. Cerebrospinal Fluid Protein Biomarkers for Alzheimer's Disease. NeuroRx. 2004;1:213–225.
3. Shaw LM, et al. Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol. 2009;65:403-13.
4. Recommendations to Update Diagnostic Criteria. The Alzheimer's Association. Website: www.alz.org/research/diagnostic_criteria.
5. Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006;23:312-28.
6. Stewart D, et al. Performance of improved CSF Aβ42 and Tau assays in differentiating AD and MCI from control subjects. Scientific Poster presented at Alzheimer's Association International Conference, 2012 (Vancouver).

For Research Use Only. Not for use in diagnostic procedures.