MSD® MULTI-ARRAY Assay System

Human (6E10) Abeta Peptide Ultra-Sensitive Kits

	1 Plate	5 Plates	20 Plates
Abeta38	K151FSE-1	K151FSE-2	K151FSE-3
Abeta40	K151FTE-1	K151FTE-2	K151FTE-3
Abeta42	K151FUE-1	K151FUE-2	K151FUE-3

MSD Neurodegenerative Disease Assays

Human (6E10) Abeta Peptide Ultra-Sensitive Kits

For use with human plasma, serum, conditioned media, and cerebrospinal fluid (CSF)

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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

Table of Contents

Contact Information

MSD Customer Service

Phone: 1-301-947-2085, 1-240-632-5885
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Beta-amyloid (Aβ) peptides derived from amyloid precursor protein (APP) are found in human CSF and have proven to be informative biomarkers with respect to neurodegeneration, especially Alzheimer's disease (AD). Amyloid plaques, the hallmark feature of the brains from AD patients upon autopsy, are enriched in Aβ42. A significant body of work supports the notion that levels of Aβ42 drop in CSF concomitant with its accumulation in the brain, first in aggregates and proto-fibrils, and ultimately in fibrils and plaques.[1,2] Quantification of Aβ42 in human CSF, especially in combination with other biomarkers, has proven to be useful in discriminating AD from other dementias and to stage AD patients with respect to the natural progression of the disease.[3,4]

Amyloid peptides are generated through successive cleavage of APP by β- and γ-secretase. Depending on the exact site of γ-secretase cleavage, benign peptides Aβ38 and Aβ40 may be produced instead of neurotoxic Aβ42. The amyloid hypothesis is the most advanced theory for the cause of AD and has been the foundation for numerous clinical trials to date.[5] This hypothesis states that oligomeric and/or aggregated forms of Aβ42 are toxic to neurons. A variety of small molecule and biological approaches have been explored in an effort to reduce the brain burden of Aβ42. The small molecule approach has largely been directed at reducing the production of Aβ42, while the biologics approach has been focused on effective clearing of Aβ42 from the brain.

The Human (6E10) Abeta Peptide Ultrasensitive Kits are sensitive and efficient means for monitoring changes in the concentrations of Aβ peptides in biological samples. These are singleplex assays that use the 6E10 monoclonal antibody as capture along with a peptide-specific detector. These assays perform well in complex matrices and can be used for the detection of Aβ peptides in samples such as human serum, plasma, conditioned neuronal cell culture medium, cell lysates, and CSF. In addition to the singleplex Human (6E10) Abeta Peptide Ultrasensitive Kits, MSD also offers a range of validated assays for measuring neurodegeneration biomarkers. Amongst these are the Aβ Peptide Panel 1 (6E10) and (4G8) kits that are validated for human CSF and mouse plasma samples.

Principle of the Assay

MSD neurodegenerative disease assays provide a rapid and convenient method for measuring the levels of Aβ peptide and protein targets within a single, small-volume sample. The Human (6E10) Abeta Peptide Ultra-Sensitive Assays are sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots in the layout shown below. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

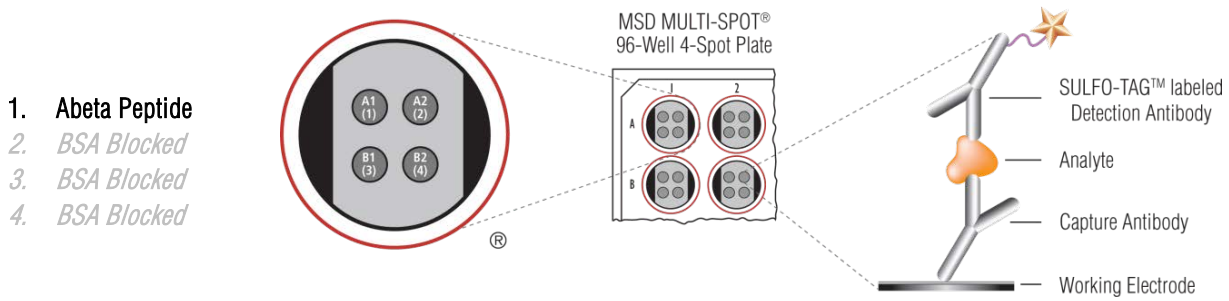


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Kit Components

Reagents Supplied With All Kits

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	20-Plate Kit	
Human Abeta Peptide Plate(s)	2–8°C	N451FSA-1	4-Spot	1	5	20	MULTI-SPOT® 96-well, 4-spot plate foil sealed, with desiccant.
Tris Wash Buffer (10X)	2–8°C	R61TX-2	200 mL	1 bottle	1 bottle		10X Tris buffered solution with surfactant for washing plates.
		R61TX-1	1000 mL			1 bottle	
Blocker A (dry powder)	RT	R93BA-4	15 g	1 bottle	1 bottle	1 bottle	Bovine serum albumin, reagent grade pure powder
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle		MSD buffer to catalyze the electrochemiluminescence reaction
		R92TC-2	200 mL			1 bottle	

Kit-Specific Components

Kits are supplied with detection antibodies and calibrators for the specific kit that was ordered.

Kit Component	Storage	Part #	Size	Quantity Supplied			Kit Name
				1-Plate Kit	5-Plate Kit	20-Plate Kit	
SULFO-TAG Anti-Aβ 38 Antibody (50X) [1]	2–8°C	D21FS-2	75 µL	1			Human (6E10) Abeta 38 Ultra-Sensitive Kit
		D21FS-3	375 µL		1	4	
SULFO-TAG Anti-Aβ 40 Antibody (50X)[1]	2–8°C	D21FT-2	75 µL	1			Human (6E10) Abeta 40 Ultra-Sensitive Kit
		D21FT-3	375 µL		1	4	
SULFO-TAG Anti-Aβ 42 Antibody (50X)[1]	2–8°C	D21FU-2	75 µL	1			Human (6E10) Abeta 42 Ultra-Sensitive Kit
		D21FU-3	375 µL		1	4	
Aβ1-40 Peptide	≤–70°C	C00OA-2	30 µL/vial	1	5	20	Synthetic peptide calibrators in diluent that mimics CSF. Analyte concentrations are provided in the lot-specific certificate of analysis.
Aβ1-38 Peptide	≤–70°C	C00NZ-2	30 µL/vial	1	5	20	
Aβ1-42 Peptide	≤–70°C	C01LB-2	30 µL/vial	1	5	20	

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1000 rpm
- ❑ Adhesive plate seals
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional safety information is available in the product safety data sheet (SDS), which can be obtained from MSD Customer Service.

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific certificate of analysis (C of A).
- Dilute calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting where necessary to avoid introduction of bubbles, and pipette to the bottom corner of empty wells.
- Shaking should be vigorous with a rotary motion between 300 and 1000 rpm.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keeping time intervals consistent between adding read buffer and reading the plate should improve inter-plate precision. Limit the time the plate is incubated with read buffer.
- No shaking is necessary after adding read buffer.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the wells.
- When running partial plates, use the sector map in the instrument or software manual to select the wells to be used. Seal the unused portion of the plate with a plate seal to avoid contaminating unused wells. You may adjust volumes proportionally when preparing detection antibody solution. After reading a partial plate, remove fluid, reseal unused sectors, return plate to its original foil pouch with desiccant pack, and seal pouch with tape. Partially used plates may be stored for up to 14 days at 2–8°C.
- Remove plate seals prior to reading the plate.

Reagent Preparation

Bring all reagents to room temperature.

The Human (6E10) Abeta Peptide Ultra-Sensitive assays may be used to measure Aβ peptides in a variety of sample types. The protocol provided below is suitable for measurement of Aβ38, Aβ40, or Aβ42 peptides in complex matrices such as serum, plasma, or conditioned medium. For measurements in human CSF, we recommend using our validated Aβ Peptide V-PLEX assays. (Visit www.mesoscale.com for kit-specific catalog numbers.) We also provide a protocol for measurement of human CSF samples in the Alternate Protocols section of this insert.

Prepare Tris Wash Buffer

MSD provides Tris Wash Buffer as a 10X stock solution. Dilute the 10X Tris Wash Buffer to 1X as shown below. Tris Wash Buffer (1X) will be used throughout the assay to make additional reagents and to wash plates. Approximately 350 mL per plate are required—more if using an automatic plate washer.

For 1 plate, combine:

- ❑ 35 mL of Tris Wash Buffer (10X)
- ❑ 315 mL of deionized water

Excess Tris Wash Buffer may be stored at room temperature in a tightly sealed container.

Prepare Blocking Solution

MSD provides Blocker A as a dry powder. Plates are blocked with a 3% Blocker A solution prepared in 1X Tris Wash Buffer.

For 1 plate, combine:

- ❑ 600 mg of Blocker A
- ❑ 20 mL of 1X Tris Wash Buffer

Prepare Assay Diluent

Assay Diluent may be used to dilute calibrators and samples for the assay if another more appropriate diluent is not available (see calibrator preparation section below for discussion). Assay Diluent is also used to prepare Detection Antibody Solution.

For 1 plate, combine:

- ❑ 3.5 mL of Blocking Solution
- ❑ 7 mL of 1X Tris Wash Buffer

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working solution is 1X.

For 1 plate, combine the following:

- ❑ 60 µL of 50X SULFO-TAG Anti-Abeta 38 Antibody or Anti-Abeta 40 Antibody or Anti-Abeta 42 Antibody
- ❑ 2940 µL of Assay Diluent

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Prepare Calibrator Dilutions

MSD supplies concentrated individual Aβ peptides as stock calibrators. For best results, Aβ peptide calibrators and samples should be prepared in a diluent that mimics the intended sample matrix as closely as possible. If a matrix-specific assay diluent is not available, you may use Assay Diluent to prepare the calibrator curve samples. Thawed stock calibrators should be mixed well by vortexing prior to use.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

1) Prepare the highest calibrator by diluting the supplied peptide calibrator with your selected assay diluent.

For Aβ 1-38 peptide:

a) Transfer 20 µL Aβ1-38 Peptide into 180 µL of assay diluent. Mix well by vortexing.

b) Transfer 30 µL of pre-diluted Aβ1-38 Peptide into 370 µL of assay diluent. Mix well by vortexing.

For Aβ 1-40 peptide:

a) Transfer 20 µL Aβ1-40 Peptide into 180 µL of assay diluent. Mix well by vortexing.

b) Transfer 20 µL of pre-diluted Aβ1-40 Peptide into 380 µL of assay diluent. Mix well by vortexing.

For Aβ 1-42 peptide:

Transfer 20 µL Aβ1-42 Peptide into 380 µL of assay diluent. Mix well by vortexing.

2) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 200 µL of assay diluent. Mix well by vortexing. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.

3) Use assay diluent as the zero calibrator.

For the lot-specific concentration of each calibrator, refer to the C of A supplied with the kit. You can also find a copy of the C of A at www.mesoscale.com.

Sample Collection and Handling

Sample collection methods and pre-analytical conditions may cause variability in measured Aβ peptide levels. MSD recommends reviewing current literature and protocols such as those proposed by the Alzheimer's Disease Neuroimaging Initiative (ADNI).[6] For serum and plasma samples, all solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze-that cycles for serum and plasma samples. Serum or plasma samples may not require a dilution prior to being tested with the Human (6E10) Abeta Peptide Ultra-Sensitive Assays.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

1. **Add Blocker A Solution:** Add 150 µL of Blocking Solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

2. **Wash and Add Sample:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 25 µL of sample or calibrator per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

4. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 150 µL of 1X Read Buffer T to each well. Read plate on MSD instrument. No incubation in read buffer is required before reading the plate.

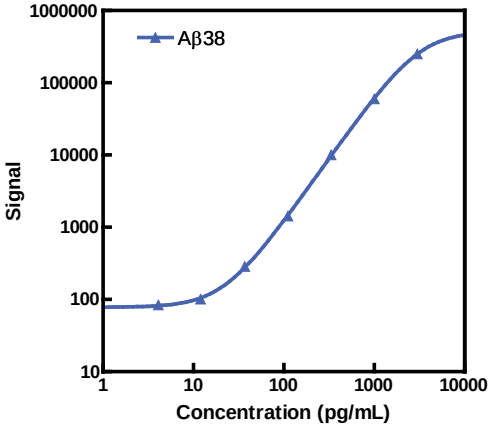
Analysis of Results

The calibration curve is modeled using least-squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification of samples without the need for multiple dilutions or repeated testing. The data displayed below were generated by MSD DISCOVERY WORKBENCH® analysis software using a 4-parameter logistic curve-fitting model (or sigmoidal dose-response) with a $1/Y^2$ weighting function. The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve.

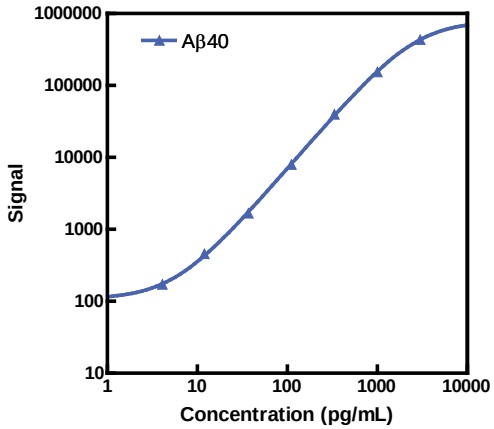
Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of 2 replicates at each calibrator level.

Typical Data

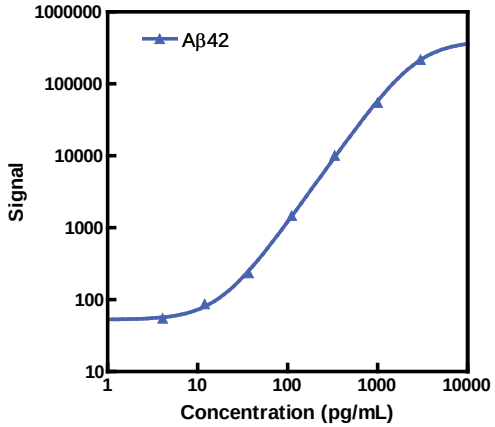
The following standard curves represent the dynamic range of each of the Human (6E10) Abeta Peptide Ultra-Sensitive assay kits. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of the standards. Calibration curves for each lot are presented in the lot-specific C of A.



Abeta 38		
Conc. (pg/mL)	Average Signal	%CV
0	74	6.7
4.1	84	13.4
12	101	4.5
37	286	5.0
111	1 425	3.1
333	10 052	3.7
1000	59 803	1.8
3000	251 041	4.8



Abeta 40		
Conc. (pg/mL)	Average Signal	%CV
0	80	11.6
4.1	172	9.0
12	458	10.1
37	1 671	6.7
111	7 987	7.5
333	39 618	7.9
1000	153 951	3.9
3000	433 862	2.0



Abeta 42		
Conc. (pg/mL)	Average Signal	%CV
0	57	12.4
4.1	55	20.8
12	87	5.8
37	235	10.2
111	1 476	7.1
333	10 089	9.9
1 000	55 089	2.5
3 000	217 379	4.4

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). Representative limits of detection for the Human (6E10) Abeta Peptide Ultra-Sensitive assay kits are provided below.

Kit Name	LLOD (pg/mL)
Human (6E10) Abeta 38 Ultra-Sensitive Kit	13
Human (6E10) Abeta 40 Ultra-Sensitive Kit	5.6
Human (6E10) Abeta 42 Ultra-Sensitive Kit	13

Assay Components

Calibrators

The assay calibrators are made using synthetic Aβ peptides.

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
Aβ38	Mouse Monoclonal	Mouse Monoclonal	A
Aβ40	Mouse Monoclonal	Mouse Monoclonal	A
Aβ42	Mouse Monoclonal	Mouse Monoclonal	A

Alternate Protocols

Human CSF Protocol

The following protocol may be used as a guidance for measuring Aβ peptides in human CSF using the Human (6E10) Abeta Peptide Ultra-sensitive kits.

Bring all reagents to room temperature.

Prepare 1X Tris Wash Buffer according to the instructions provided in the Reagent Preparation section of this insert.

Prepare 10% Blocker A Solution

MSD provides Blocker A as a dry powder. Plates are blocked with a 10% Blocker A solution prepared in 1X Tris Wash Buffer.

For 1 plate, combine:

- ❑ 2 g of Blocker A (dry powder)
- ❑ 20 mL of 1X Tris Wash Buffer

Prepare Antibody Diluent

For 1 plate, combine:

- ❑ 500 µL of 10% Blocker A Solution
- ❑ 4.5 mL of 1X Tris Wash Buffer

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working solution is 1X.

For 1 plate, combine the following:

- ❑ 60 µL of 50X SULFO-TAG Anti-Abeta 38 Antibody or Anti-Abeta 40 Antibody or Anti-Abeta 42 Antibody
- ❑ 2940 µL of Antibody Diluent

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Prepare Calibrator Dilutions

MSD supplies concentrated individual Aβ peptides as stock calibrators. For best results, Aβ peptide calibrators and samples should be prepared in a diluent that mimics the intended sample matrix as closely as possible. The following protocol is recommended for measurement of Aβ peptides in CSF.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

1) Prepare the highest calibrator by diluting the supplied peptide calibrator with your selected assay diluent.

For Aβ 1-38 peptide:

a) Transfer 20 µL Aβ1-38 Peptide into 180 µL of 10% Blocker A Solution. Mix well by vortexing.

b) Transfer 30 µL of pre-diluted Aβ1-38 Peptide into 370 µL of 10% Blocker A Solution. Mix well by vortexing.

For Aβ 1-40 peptide:

Transfer 15 µL Aβ1-40 Peptide into 885 µL of 10% Blocker A Solution. Mix well by vortexing.

For Aβ 1-42 peptide:

Transfer 20 µL Aβ1-42 Peptide into 380 µL of 10% Blocker A Solution. Mix well by vortexing.

2) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 200 µL of 10% Blocker A Solution. Mix well by vortexing. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.

3) Use 10% Blocker A Solution as the zero calibrator.

For the lot-specific concentration of each calibrator, refer to the C of A supplied with the kit. You can also find a copy of the C of A at www.mesoscale.com.

Sample Collection and Handling

Sample collection methods and pre-analytical conditions may cause variability in measured Aβ peptide levels. MSD recommends reviewing current literature and protocols such as those proposed by the Alzheimer's Disease Neuroimaging Initiative (ADNI).[6] Samples should be used immediately, or frozen in aliquots and stored at ≤-70°C until needed. Repeated freeze–thaw of samples is not recommended. Evaluate sample stability under the selected method as needed. CSF samples may not require dilution prior to testing in the assay.

Protocol

1. **Add Blocker A Solution:** Add 25 µL of 10% Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 30 minutes.

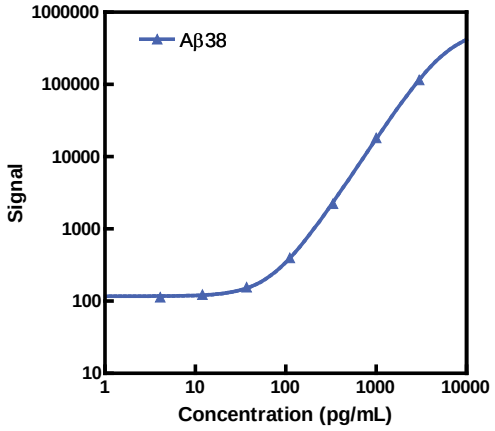
2. **Add Sample:** Do not wash plate before addition of calibrators or samples. Add 25 µL of sample or calibrator per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

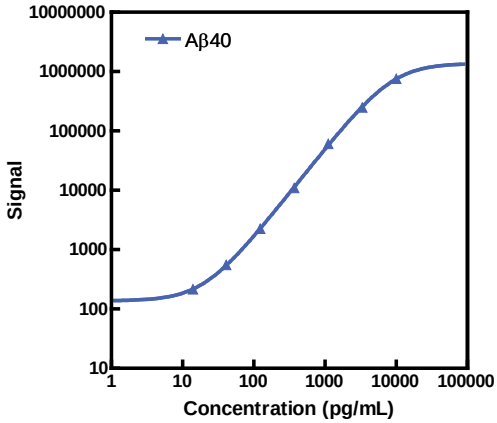
4. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of 1X Tris Wash Buffer. Add 150 µL of 2X Read Buffer T to each well. Read plate on MSD instrument. No incubation in read buffer is required before reading the plate.

Typical Data

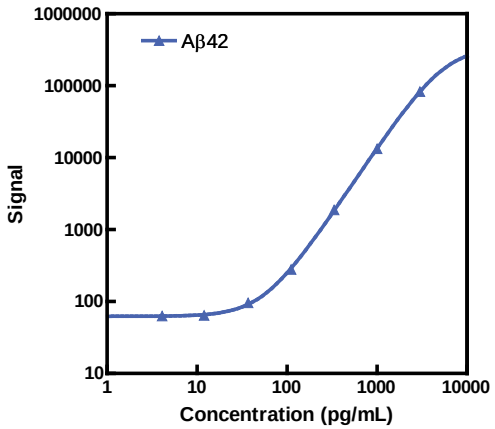
Representative standard curves for each assay tested using the human CSF protocol is shown below. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of the standards.



Abeta 38		
Conc. (pg/mL)	Average Signal	%CV
0	114	8.1
4.1	113	8.7
12	123	4.5
37	156	5.7
111	398	6.4
333	2 229	9.1
1000	18 131	4.0
3000	115 683	4.3



Abeta 40		
Conc. (pg/mL)	Average Signal	%CV
0	95	3.8
14	215	7.0
41	552	5.5
123	2 243	5.4
370	10 912	7.8
1 111	60 658	5.8
3 333	247 683	3.2
10 000	753 688	4.0



Abeta 42		
Conc. (pg/mL)	Average Signal	%CV
0	58	2.0
4.1	63	11.5
12	64	11.8
37	96	7.7
111	279	8.3
333	1 883	1.0
1 000	13 293	1.2
3 000	82 671	8.3

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). Representative limits of detection for the Human (6E10) Abeta Peptide Ultra-Sensitive assay kits are provided below.

Kit Name	LLOD (pg/mL)
Human (6E10) Abeta 38 Ultra-Sensitive Kit	32
Human (6E10) Abeta 40 Ultra-Sensitive Kit	18
Human (6E10) Abeta 42 Ultra-Sensitive Kit	34

References

1. Albert MS, et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011 May;7(3):270-9.
2. Blennow K, et al. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol. 2010 Mar;6(3):131-44.
3. Gabelle A, et al. Correlations between soluble α/β forms of amyloid precursor protein and Aβ38, 40, and 42 in human cerebrospinal fluid. Brain Res. 2010 Oct 21;1357:175-83.
4. Jack CR Jr, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. Lancet Neurol. 2013 Feb;12(2):207-16.
5. Karran E, et al. The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics. Nat Rev Drug Discov. 2011 Aug 19;10(9):698-712.
6. Alzheimer's Disease Neuroimaging Initiative Procedures Manuals. Website: http://www.adni-info.org/Scientists/ProceduresManuals.aspx.

Summary Protocol

Human (6E10) Abeta Peptide Ultra-Sensitive Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human (6E10) Abeta Peptide Ultra-Sensitive assays.

Sample and Reagent Preparation

Prepare Tris Wash Buffer.

Prepare Blocker A solution.

Prepare assay diluent and antibody dilution buffer.

Prepare samples.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in antibody dilution buffer.

Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with shaking for 1 hour.

Step 2: Wash and Add Sample

Wash plate 3 times with at least 150 µL/well of Tris Wash Buffer.

Add 25 µL/well of calibrators or samples.

Incubate at room temperature with shaking for 1 hour.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with at least 150 µL/well of Tris Wash Buffer.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with shaking for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with at least 150 µL/well of Tris Wash Buffer.

Add 150 µL/well of 1X Read Buffer T.

Analyze plate on MSD instrument.

Plate Diagram