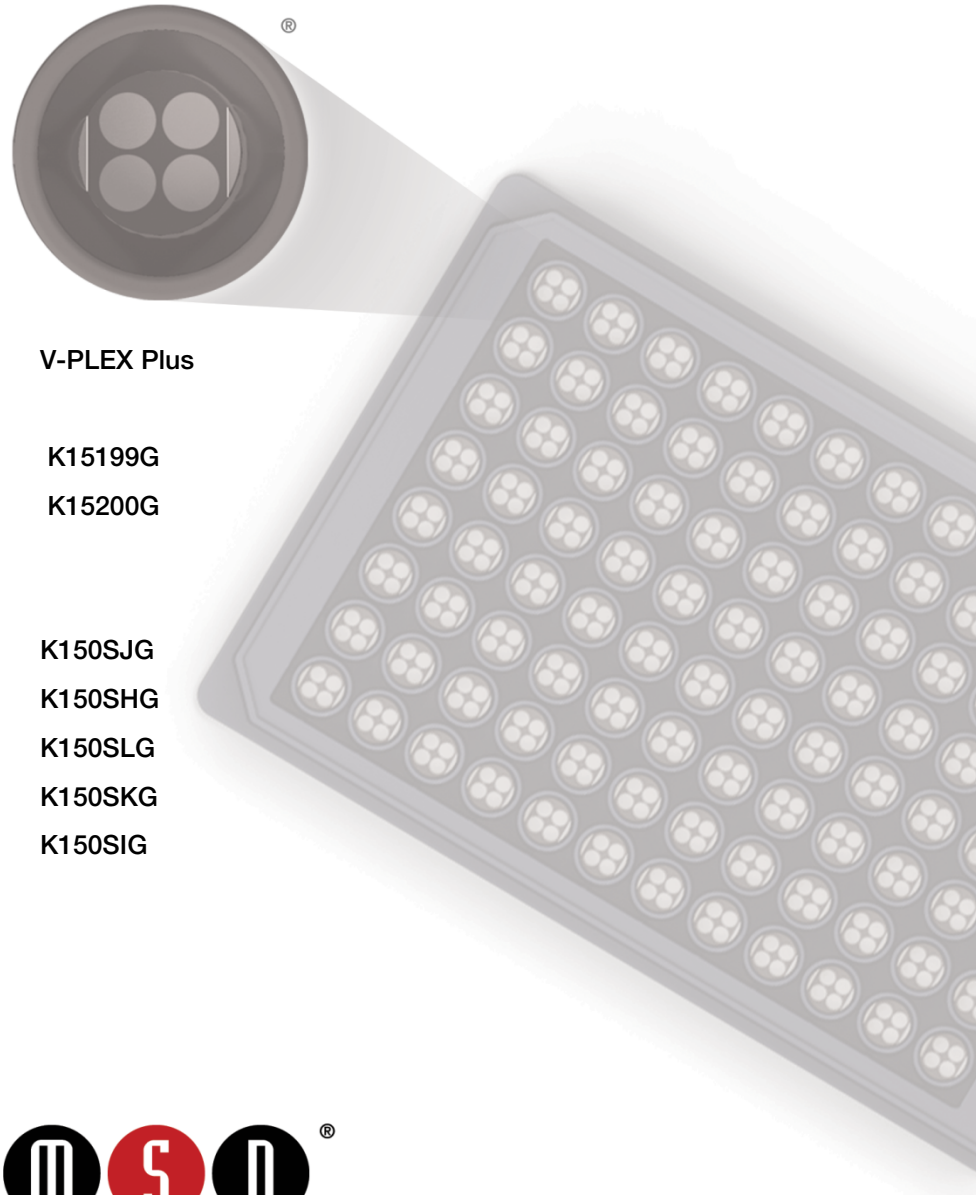
MSD® MULTI-SPOT Assay System

Aβ Peptide Panel 1 Kits

Aβ38, Aβ40, Aβ42

V-PLEX®



	V-PLEX®	V-PLEX Plus
Multiplex Kits		
Aβ Peptide Panel 1 (4G8)	K15199E	K15199G
Aβ Peptide Panel 1 (6E10)	K15200E	K15200G
Singleplex Kits		
Aβ40 Peptide (4G8)	K150SJE	K150SJG
Aβ38 Peptide (4G8)	K150SHE	K150SHG
Aβ42 Peptide (4G8)	K150SLE	K150SLG
Aβ40 Peptide (6E10)	K150SKE	K150SKG
Aβ38 Peptide (6E10)	K150SIE	K150SIG

MSD Neurodegenerative Disease Assays

Aβ Peptide Panel 1 Kits

Aβ40, Aβ38, Aβ42

For use with human cerebrospinal fluid (CSF), rodent CSF, rodent plasma/serum, cell culture supernatants, and tissue homogenates.

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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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 that levels of Aβ42 drop in CSF concomitantly 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; thus these biomarkers have proven useful in AD research.[3,4]

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.

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. Clinical trials to date have included small molecules that inhibit β-secretase or those that modulate γ-secretase to favor production of Aβ38 and Aβ37 over Aβ42 and Aβ40.[6] The Aβ Peptide Panel 1 provides a sensitive and efficient means to measure changes in concentrations of the Aβ peptides in samples during clinical research with these small molecules. While the Aβ Peptide Panel 1 (6E10) and (4G8) kits are validated for human CSF, they are also compatible with other sample types including conditioned neuronal cell culture medium, cell lysates, and tissue homogenates including mouse brain, and thus provide a platform for use in early discovery through pre-clinical and clinical research studies. The Aβ Peptide Panel 1 (4G8) Kit has also been validated for mouse EDTA plasma.

Principle of the Assay

MSD neurodegenerative disease assays provide a rapid and convenient method for measuring the levels of peptide and protein targets within a single, small-volume sample. The assays in the Aβ Peptide Panel 1 are sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots as shown in the layout below. Assay kits are provided with 4-spot MULTI-SPOT® plates (Figures 1 and 2). 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 creates 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 (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample. V-PLEX assay kits have been validated according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J. W. Lee, et al.[9]

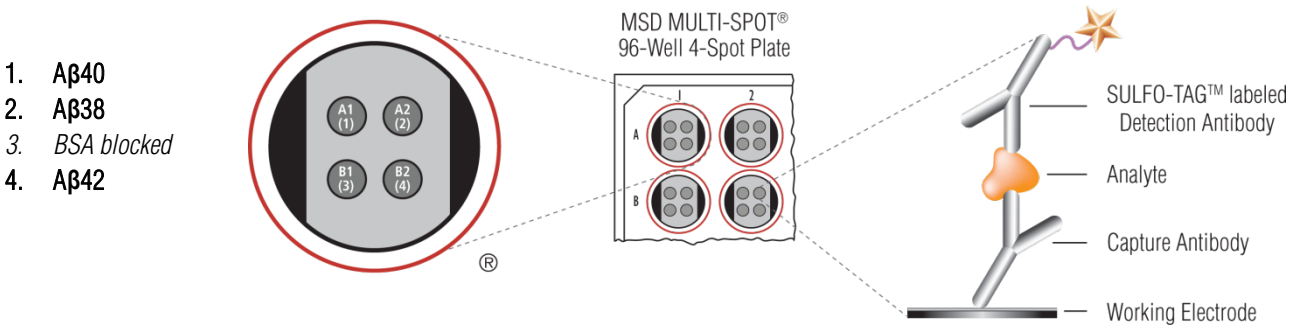


Figure 1. Spot diagram showing placement of analyte capture antibodies for the Aβ Peptide Panel 1 and Aβ38 singleplex kits. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

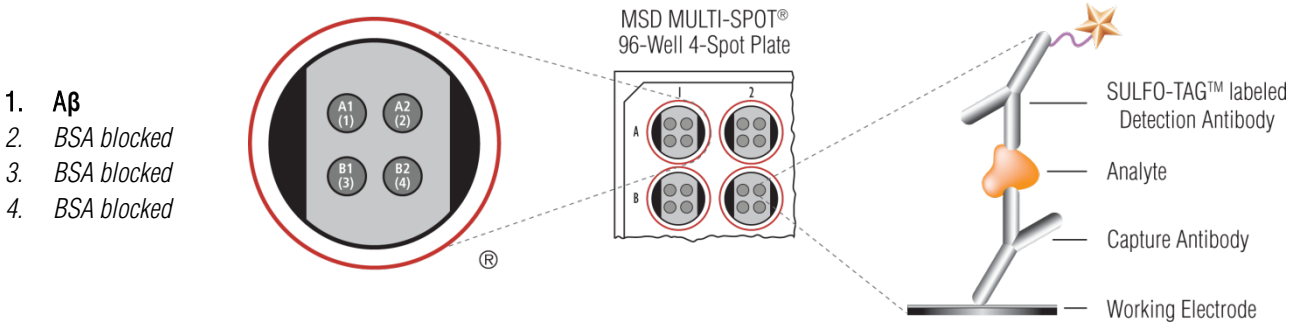


Figure 2. Spot diagram showing placement of analyte capture antibodies for Aβ40 and Aβ42 singleplex plates. Aβ40 and Aβ42 singleplex kits are provided on 4-spot plates with analyte capture antibody coated on spot A1. Spots A2, B1, and B2 are BSA blocked.

Kit Components

Aβ Peptide Panel 1 assays are available as a 4-spot multiplex kit and as single assay kits. All V-PLEX kits are provided with pre-coated plates, calibrator, detection antibodies, and reagents. V-PLEX Plus kits include additional items (controls, wash buffer, and plate seals). See below for details on the components.

See the **Catalog Numbers** section for a comprehensive list of all kits.

Reagents Supplied With All Kits

Table 1. Reagents that are supplied with V-PLEX and V-PLEX Plus Kits

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	
Diluent 35	2–8°C	R50AE-3	30 mL	1 bottle			Diluent for samples and calibrator which mimics human CSF; contains proteins and preservatives.
		R50AE-2	150 mL		1 bottle	5 bottles	
Diluent 100	2–8°C	R50AA-4	50 mL	1 bottle			Diluent for detection antibody; contains protein, blockers, and preservatives.
					1 bottle	5 bottles	
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	Buffer to catalyze the electro-chemiluminescence reaction.

Kit-Specific Components

Table 2. Components that are supplied with specific kits

Plates	Storage	Part #	Size	Quantity Supplied			Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	
Aβ Peptide Panel 1 (6E10) Plate	2–8°C	N45197A-1	4-spot	1	5	25	96-well plate, foil sealed, with desiccant.
Aβ Peptide Panel 1 (4G8) Plate	2–8°C	N45199A-1	4-spot	1	5	25	
Aβ40 Peptide Plate	2–8°C	N450OAA-1	4-spot	1	5	25	
Aβ38 Peptide Plate	2–8°C	N45197A-1	4-spot	1	5	25	
Aβ42 Peptide Plate	2–8°C	N451LBA-1	4-spot	1	5	25	

Table 3. Kits are supplied with individual calibrators for each assay ordered

Calibrators	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	
Aβ1-40 Peptide (6E10)	≤–70°C	C00OA-2	30 µL/vial	1	5	25	Synthetic peptide calibrators in diluent that mimics human CSF. Analyte concentrations are provided in the lot-specific certificate of analysis (COA).
Aβ1-38 Peptide (6E10)	≤–70°C	C00NZ-2	30 µL/vial	1	5	25	
Aβ1-42 Peptide (6E10)	≤–70°C	C01LB-2	30 µL/vial	1	5	25	
Aβ1-40 Peptide (4G8)	≤–70°C	C002Z-2	30 µL/vial	1	5	25	
Aβ1-38 Peptide (4G8)	≤–70°C	C002Y-2	30 µL/vial	1	5	25	
Aβ1-42 Peptide (4G8)	≤–70°C	C003A-2	30 µL/vial	1	5	25	

Table 4. Kits are supplied with detection antibodies for the specific kit that was ordered

SULFO-TAG Detection Antibody	Storage	Catalog #	Size	Quantity Supplied 1 Plate Kit	5 Plate Kit	25 Plate Kit	Description
Anti-Aβ 4G8 Antibody (50X)*	2–8°C	D20RQ-2	75 µL	1			SULFO-TAG conjugated antibody specific for human and rodent Aβ peptides.
		D20RQ-3	375 µL		1	5	
Anti-Aβ 6E10 Antibody (50X)*	2–8°C	D21LB-2	75 µL	1			SULFO-TAG conjugated antibody specific for human Aβ peptides.
		D21LB-3	375 µL		1	5	

*SULFO-TAG conjugated detection antibodies should be stored in the dark.

Table 5. Kits that include the Aβ40 assay are supplied with Aβ40 Blocker

Blocker	Storage	Catalog #	Size	Quantity Supplied 1 Plate Kit	5 Plate Kit	25 Plate Kit	Description
Aβ40 Blocker	2–8°C	R93BJ-1	40 µL	1			Blocking reagent for measuring CSF samples containing high abundance of Aβ40 peptide; not for use with serum and plasma.
		R93BJ-2	200 µL		1	5	

V-PLEX Plus Kits: Additional Components

Table 6. Additional components that are supplied with V-PLEX Plus Kits

Reagents	Storage	Catalog #	Size	Quantity Supplied 1 Plate Kit	5 Plate Kit	25 Plate Kit	Description
Neurodegeneration Control 1 (6E10)*	≤–70°C	C41LB-1	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls in diluent that mimics human CSF. The concentrations of the analytes are provided in the lot-specific COA..
Neurodegeneration Control 2 (6E10)*	≤–70°C	C41LB-1	1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 3 (6E10)*	≤–70°C	C41LB-1	1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 1 (4G8)**	≤–70°C	C40RQ-1	1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 2 (4G8)**	≤–70°C	C40RQ-1	1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 3 (4G8)**	≤–70°C	C40RQ-1	1 vial	1 vial	5 vials	25 vials	
Wash Buffer (20X)	RT	R61AA-1	100 mL	1 bottle	1 bottle	5 bottles	20-fold concentrated phosphate buffered solution with surfactant.
Plate Seals	-	-	-	3	15	75	Adhesive seals for sealing plates during incubations.

*Provided as components within Neurodegeneration Control Pack 1 (6E10) (catalog # C41LB-1)
**Provided as components within Neurodegeneration Control Pack 1 (4G8) (catalog # C40RQ-1)

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 500–1,000 rpm
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog # R61AA-1 (included in V-PLEX Plus kit)
- ❑ Adhesive plate seals (3 per plate included in V-PLEX Plus kits)
- ❑ Deionized water
- ❑ Vortex mixer

Optional Materials and Equipment

- ❑ Neurodegeneration Control Pack 1 (6E10), available for separate purchase from MSD, catalog # C41LB-1 (included in V-PLEX Plus kit)
- ❑ Neurodegeneration Control Pack 1 (4G8), available for separate purchase from MSD, catalog # C40RQ-1 (included in V-PLEX Plus kit)
- ❑ Centrifuge (for sample preparation)

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 product-specific safety information is available in the applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific COA.
- Assay incubation steps should be performed between 20–26°C to achieve the most consistent signals between runs.
- Prepare 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 T may interfere with signal detection.
- Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding 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 plate.
- Remove the plate seals prior to reading the plate.
- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors (see sector map in instrument and software manuals) to avoid contaminating unused wells. Remove all seals before reading. Partially used plates may be sealed and stored up to 7 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature. Diluted calibrator, controls, and samples should be prepared during the blocking step and used within one hour of preparation. Thorough mixing of stock and diluted kit reagents is required.

Prepare Calibrator Dilutions

MSD supplies individual Aβ peptide calibrators at a concentration that is 40-fold higher than the recommended highest standard.

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 Diluent 35. Mix well by vortexing.

   Instructions for Aβ Peptide Panel 1 multiplex kits (Figure 3):

   Transfer 10 µL of Aβ1-40 Peptide, 10 µL Aβ1-38 Peptide, and 10 µL Aβ1-42 Peptide into 370 µL of Diluent 35.

   Or

   Instructions for Aβ singleplex assay kits (Figure 4):

   Add 10 µL of the supplied peptide calibrator to 390 µL of Diluent 35.

2) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 300 µL of Diluent 35. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.

3) Use Diluent 35 as the zero calibrator.

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

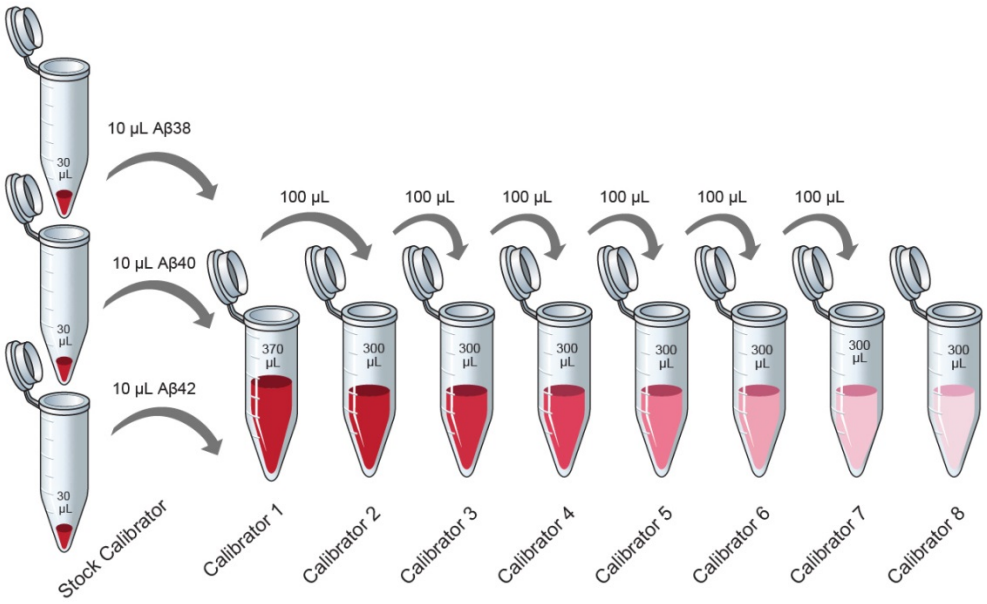


Figure 3. Preparation of calibrator solutions for Aβ Peptide Panel 1 multiplex kits

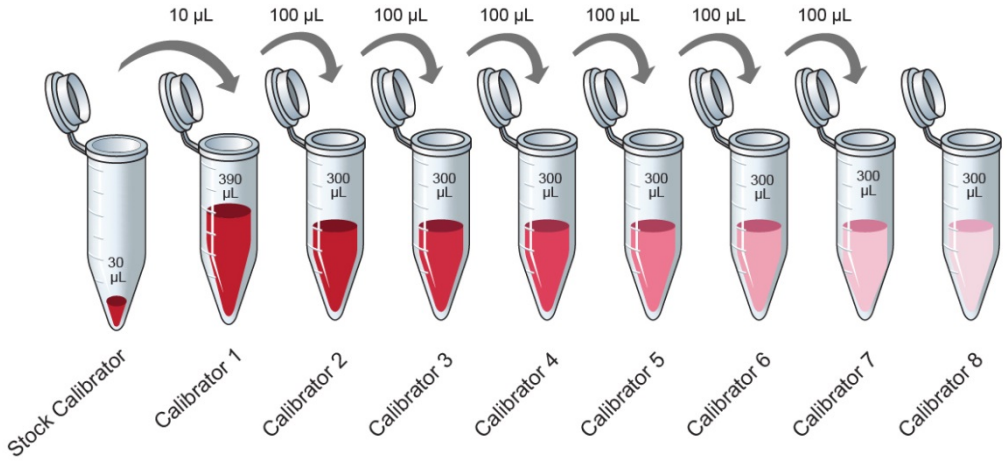


Figure 4. Preparation of calibrator solutions for Aβ singleplex assay kits

Sample Collection and Handling

CSF 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).[10-12] Samples described herein were clarified through a single centrifugation step at 1,200 rcf for 10 minutes at 2–8˚C. 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.

Dilute Samples

Human CSF samples: MSD recommends a minimum 2-fold sample dilution; however depending on the sample set under investigation, a higher dilution factor may be needed to maximize matrix tolerance. Mix thawed sample well by vortexing, then dilute with Diluent 35. For example, to dilute 2-fold, add 60 µL of sample to 60 µL of Diluent 35. Mix diluted sample well by vortexing.

Mouse plasma samples: Mouse samples may be tested with the Aβ Peptide Panel 1 (4G8) kit only. MSD recommends a 4-fold dilution for evaluation of mouse EDTA plasma. Mix thawed samples well by vortexing, then dilute with Diluent 35. For example, to dilute 4-fold, add 30 µL of sample to 90 µL of Diluent 35.

The Aβ Peptide Panel 1 kits exhibit good dilution linearity; you may conserve CSF or plasma sample volume by using a higher sample dilution. Please see the dilution linearity section for representative data.

Prepare Controls

Three levels of multi-analyte frozen liquid controls are available for separate purchase from MSD in the Neurodegeneration Control Pack 1, catalog # C41LB-1 (6E10) or C40RQ-1 (4G8). (Controls are included only in V-PLEX Plus Kits.) The controls are prepared by spiking known levels of synthetic Aβ peptides into a diluent that mimics human CSF.

Thaw controls at room temperature and mix well by vortexing. Dilute controls 2-fold in Diluent 35 and mix well by vortexing. For the lot-specific concentrations of each analyte in the control pack, refer to the supplied COA. You can also find a copy of the COA at www.mesoscale.com.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use.

For kits that include Aβ40 assay, the Aβ40 Blocker may be included in the working detection antibody solution at a final concentration of 1X. For sample types that are expected to have low levels of Aβ40 peptide, Aβ40 blocker may be omitted. Omission of Aβ40 blocker may result in high signals of the Aβ40 controls, close to the upper limit of quantification.

Detection Antibody Solution with Aβ40 Blocker

For one plate, combine the following:

- ❑ 60 µL of 50X SULFO-TAG Anti-Aβ 4G8 Antibody or Anti-Aβ 6E10 Antibody
- ❑ 30 µL of Aβ40 Blocker
- ❑ 2,910 µL of Diluent 100

Detection Antibody Solution without Aβ40 Blocker

For one plate, combine the following:

- ❑ 60 µL of 50X SULFO-TAG Anti-Aβ 4G8 Antibody or Anti-Aβ 6E10 Antibody
- ❑ 2,940 µL of Diluent 100

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution in the V-PLEX Plus kit. The working solution is 1X. PBS + 0.05% Tween-20 can be used instead.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

1X MSD Wash Buffer can be stored at room temperature for up to two weeks.

Prepare Read Buffer T

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

For one 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 one month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figures 1 and 2) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates may be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Add Blocker

- ❑ Add 150 µL of Diluent 35 to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

STEP 2: Wash and Add Detection Antibody Solution and Sample

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well.
- ❑ Add 25 µL of prepared samples, calibrators or controls per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer T is not required before reading the plate.

Validation

MSD's V-PLEX products are validated following fit-for-purpose principles[9] and MSD design control procedures. Three independently-built kit lots were included in the validation of the Aβ Peptide Panel 1 (6E10) and (4G8) kits. Accuracy and precision of calibrators and controls were determined from multiple test runs conducted over several days by multiple analysts. Assay specificity, tolerance to interferences, robustness, and stability were also evaluated. The validation methods used are described below.

- **Sensitivity**

  The upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) were determined through measurement of samples created by spiking known amounts of calibrator into assay diluent. The accuracy and precision of these measurements was evaluated across multiple kit lots.

- **Precision**

  Precision is presented as the coefficient of variance (CV). The typical specification for precision is a concentration CV of less than 25% for controls on both intra- and inter-day runs.

- **Dilution Linearity, and Spike Recovery**

  Spike recovery and dilution linearity were assessed across multiple kit lots.

- **Specificity**

  Assay specificity and tolerance to interferences were evaluated across multiple lots using peptides within the kit, and a panel of other analytes of interest.

- **Robustness and Stability**

  The robustness of the protocol was evaluated to examine the boundaries of the selected incubation times. Assay component robustness was assessed through freeze–thaw testing and stability studies for calibrators, antibodies, and controls. The validation program includes a real–time stability study with scheduled performance evaluations of complete kits for up to 54 months from date of manufacture.

Representative data from the validation studies are presented in the following sections. The calibration curve and measured limits of detection for each lot can be found in the lot-specific COA that is included with each kit and available for download at www.mesoscale.com.

Analysis of Results

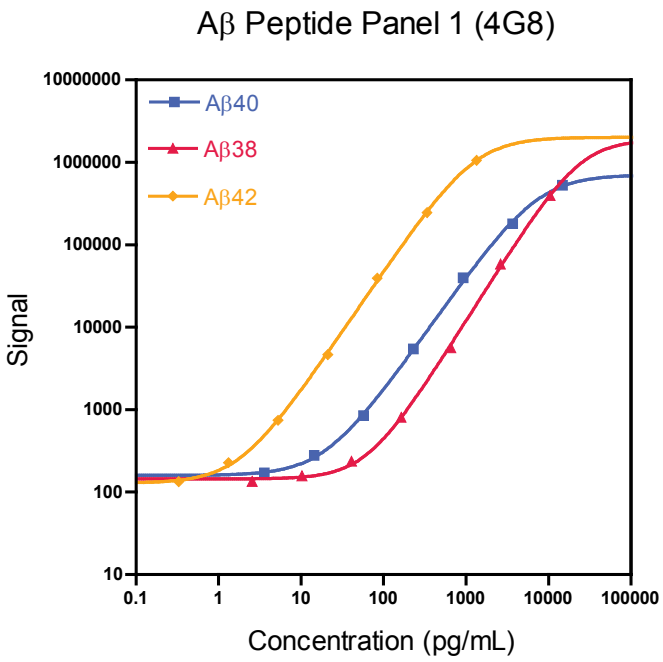
The calibration curves used to calculate analyte concentrations were established by fitting the signals from the calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve. Analyte concentrations were determined from the ECL signals by back-fitting to the calibration curve. These assays have a wide dynamic range (4 logs), which allows accurate quantification of samples without the need for multiple dilutions or repeated testing. The calculations to establish calibration curves and determine concentrations were carried out using the MSD DISCOVERY WORKBENCH® analysis software.

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

Typical Data

Calibration curve accuracy and precision were assessed for three kit lots. Representative data from one kit lot are presented below. The average intra-plate signal CVs were typically less than 8%. Calibration curves for each lot are presented in the lot-specific COA.

Figure 5. Typical calibration curves and representative data for the Aβ Peptide Panel 1 (4G8) assay



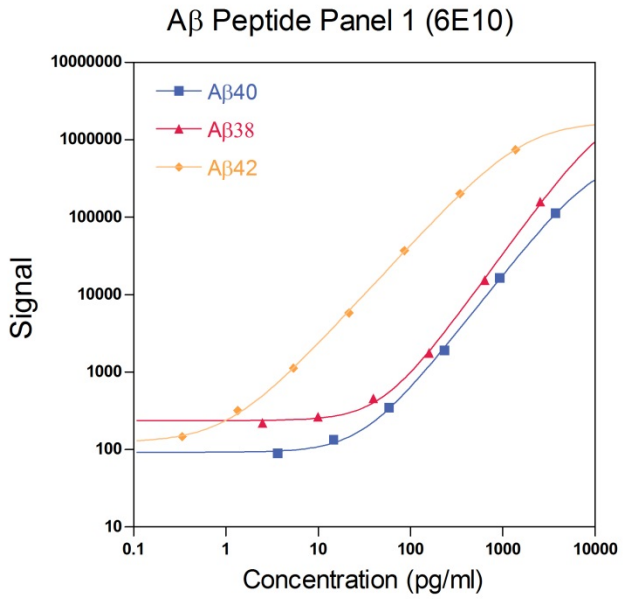
Aβ40[1]		
Assigned Conc. (pg/mL)	Average Signal	%CV
14,825	528,168	5.9
3,706	179,871	6.9
927	39,760	7.4
232	5,451	7.8
57.9	850	7.5
14.9	278	8.4
3.62	171	4.4
0	148	5.9

Aβ38[1]		
Assigned Conc. (pg/mL)	Average Signal	%CV
10,500	396,928	3.2
2,625	58,861	5.2
656	5,659	5.6
164	810	4.0
41.0	238	4.6
10.3	158	6.1
2.56	135	5.4
0	132	5.3

Aβ42[1]		
Assigned Conc. (pg/mL)	Average Signal	%CV
1,348	1,055,597	2.8
337	246,458	3.1
84.2	39,402	4.9
21.1	4,663	5.1
5.26	747	5.6
1.32	229	8.4
0.329	134	6.4
0	110	8.2

[1]See the kit-specific COA for calibration curve concentrations, specifications, and quality control data.

Figure 6. Typical calibration curves and representative data for the Aβ Peptide Panel 1 (6E10) assay



Aβ40[2]		
Assigned Conc. (pg/mL)	Average Signal	%CV
14,900	391,584	5.3
3,725	113,481	6.8
931	16,502	8.4
233	1,908	8.5
58.2	352	7.5
14.6	134	4.4
3.64	90	5.3
0	77	5.0

Aβ38[2]		
Assigned Conc. (pg/mL)	Average Signal	%CV
10,200	952,636	3.4
2,550	158,685	5.4
638	15,447	7.8
159	1,777	5.0
39.8	460	7.6
9.96	266	5.1
2.49	222	7.8
0	212	6.9

Aβ42[2]		
Assigned Conc. (pg/mL)	Average Signal	%CV
1,380	744,788	2.3
345	201,808	2.8
86.3	37,005	3.9
21.6	5,831	4.6
5.39	1,127	3.4
1.35	319	2.9
0.337	147	4.2
0	93	7.6

[2]See the kit-specific COA for calibration curve concentrations, specifications, and quality control data.

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). The LLODs shown below were calculated based on 50 runs across 3 kit lots for each kit product.

The ULOQ is the highest concentration at which the %CV of the calculated concentration is <20% and the percent recovery of the standard is within 80–120% of the known value.

The LLOQ is the lowest concentration at which the %CV of the calculated concentration is <20% and the percent recovery of the standard is within 80–120% of the known value.

The quantitative range of the assay lies between the LLOQ and ULOQ.

The LLOQ and ULOQ are verified for each kit lot and the results are provided in the lot-specific COA that is included with each kit and available at www.mesoscale.com.

Table 7. LLOD, LLOQ, and ULOQ for each analyte in the Aβ Peptide Panel 1 Kits

Kit Name	Analyte	Median LLOD (pg/mL)	LLOD Range (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
Aβ Peptide Panel 1 (4G8)	Aβ40	5.41	3.72–42.6	20.0	6,000
	Aβ38	22.2	13.8–57.7	60.0	7,500
	Aβ42	0.516	0.198–1.15	2.50	1,270
Aβ Peptide Panel 1 (6E10)	Aβ40	9.97	7.26–12.2	50.0	7,000
	Aβ38	16.7	7.59–24.3	60.0	8,480
	Aβ42	0.368	0.220–0.537	3.13	1,270

Precision

Neurodegeneration Controls 1, 2, and 3 were made by spiking Aβ peptides into diluent that mimics human CSF. CSF Controls 1, 2, and 3 were made by spiking peptides into human CSF. Peptides were spiked at three levels within the quantitative range of the assay to create controls 1, 2, and 3. Analyte levels were measured by five analysts using a minimum of two replicates on ≥37 runs over eight months across three kit lots. Results are shown below. While the typical specification for precision is a concentration CV of less than 25% for controls on both intra- and inter-day runs, for this panel, the data indicates that for most assays the CVs are below 10%.

Average intra-plate %CV is the average %CV of the control replicates within an individual run.

Inter-plate %CV is the variability of controls across ≥37 runs.

Inter-lot %CV is the variability of controls across three kit lots.

Table 8. Intra-run and Inter-run %CVs in the Aβ Peptide Panel 1 Kits

Kit Name	Analyte	Control	Ave. Conc. (pg/mL)	Ave. Intra-plate %CV	Ave. Inter-plate Conc. %CV	Inter-Kit Lot Conc. %CV
Aβ Peptide Panel 1 (4G8)	Aβ40	Neurodegeneration Control 1 (4G8)	9,071	6.8	9.7	7.9
		Neurodegeneration Control 2 (4G8)	3,036	5.3	8.3	7.0
		Neurodegeneration Control 3 (4G8)	570	5.2	11.1	9.1
		CSF Control 1	8,963	5.1	10.7	9.6
		CSF Control 2	3,019	5.2	10.5	9.9
		CSF Control 3	1,865	5.7	9.6	7.9
	Aβ38	Neurodegeneration Control 1 (4G8)	2,754	4.8	7.0	4.7
		Neurodegeneration Control 2 (4G8)	694	4.9	9.9	8.5
		Neurodegeneration Control 3 (4G8)	183	5.2	17.6	18.0
		CSF Control 1	4,977	4.3	6.9	3.6
		CSF Control 2	1,321	4.5	11.7	11.4
		CSF Control 3	609	4.2	13.0	13.0
	Aβ42	Neurodegeneration Control 1 (4G8)	1,303	2.3	7.5	7.5
		Neurodegeneration Control 2 (4G8)	495	2.2	7.7	7.3
		Neurodegeneration Control 3 (4G8)	206	2.2	8.2	7.6
		CSF Control 1	1,804	3.5	6.8	6.5
		CSF Control 2	497	2.4	7.6	6.4
		CSF Control 3	261	2.9	7.5	6.6
Aβ Peptide Panel 1 (6E10)	Aβ40	Neurodegeneration Control 1 (6E10)	9,957	5.5	7.1	6.3
		Neurodegeneration Control 2 (6E10)	3,236	4.4	6.0	3.6
		Neurodegeneration Control 3 (6E10)	615	4.3	7.8	3.8
		CSF Control 1	9,415	4.4	6.9	5.5
		CSF Control 2	3,111	4.2	7.9	6.1
		CSF Control 3	1,933	4.9	8.3	4.2
	Aβ38	Neurodegeneration Control 1 (6E10)	3,044	4.2	7.1	3.8
		Neurodegeneration Control 2 (6E10)	768	3.9	6.2	1.2
		Neurodegeneration Control 3 (6E10)	197	4.4	9.5	7.8
		CSF Control 1	5,276	3.1	7.2	5.3
		CSF Control 2	1,471	3.6	6.8	0.6
		CSF Control 3	705	4.0	7.6	3.0
	Aβ42	Neurodegeneration Control 1 (6E10)	1,440	2.2	7.0	7.0
		Neurodegeneration Control 2 (6E10)	532	2.0	6.5	6.1
		Neurodegeneration Control 3 (6E10)	216	2.5	7.4	5.9
		CSF Control 1	1,925	3.0	8.4	9.2
		CSF Control 2	513	2.4	8.3	9.0
		CSF Control 3	263	2.7	8.8	8.0

Dilution Linearity

To assess linearity, CSF samples collected from individual patients according to accepted protocols[10] and mouse EDTA plasma samples were diluted 2-fold, 4-fold, and 8-fold before testing. Percent recovery at each dilution was calculated by dividing the dilution-adjusted concentration by the dilution-adjusted concentration at 2-fold dilution (for human CSF samples) or 4-fold (for mouse plasma samples). The average percent recovery shown below is based on samples within the quantitative range of the assay.

$$\%\ Recovery = \frac{(measured\ concentration \times dilution\ factor)}{(measured\ concentration\ at\ 2\text{–}fold\ dilution \times 2)} \times 100$$

Figure 7. Dilution linearity in human CSF and mouse plasma samples for the Aβ Peptide Panel 1 Kits

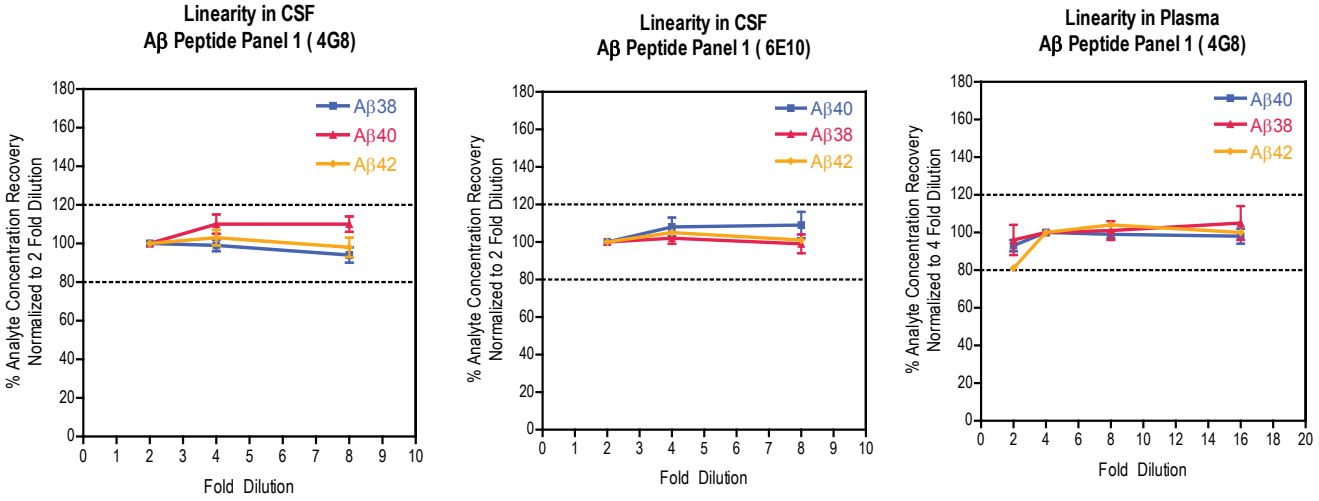


Table 9. Analyte percent recovery at various dilutions in each sample type in the Aβ Peptide Panel 1 Kits

			Aβ40		Aβ38		Aβ42	
Kit Name	Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Aβ Peptide Panel 1 (4G8)	Human CSF (N=10)	2	100	N/A	100	N/A	100	N/A
		4	110	103–118	99	99–93	103	103–103
		8	110	105–119	94	94–86	98	103–98
	Mouse Plasma (N=10)	2	93	89–98	96	96–84	81	105–81
		4	100	N/A	100	N/A	100	N/A
		8	99	97–104	101	101–95	104	108–104
		16	98	94–104	105	105–94	100	119–100
Aβ Peptide Panel 1 (6E10)	Human CSF (N=10)	2	100	N/A	100	N/A	100	N/A
		4	108	101–114	102	102–96	105	106–105
		8	109	101–125	99	99–92	101	107–101

Spike Recovery

Spike and recovery measurements of different sample types were evaluated throughout the quantitative range of the assays. Multiple individual human CSF or mouse EDTA plasma samples were spiked with calibrators at two levels (high and low) and then diluted 2-fold (for human CSF samples) or 4-fold (for mouse plasma samples). The average % recovery for each sample type is reported along with %CV and % recovery range. The expected concentration is the sum of the measured endogenous concentration and the spiked calibrator concentration.

$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} \times 100$$

Table 10. *Spike and Recovery measurements of different sample types in the Aβ Peptide Panel 1 Kits*

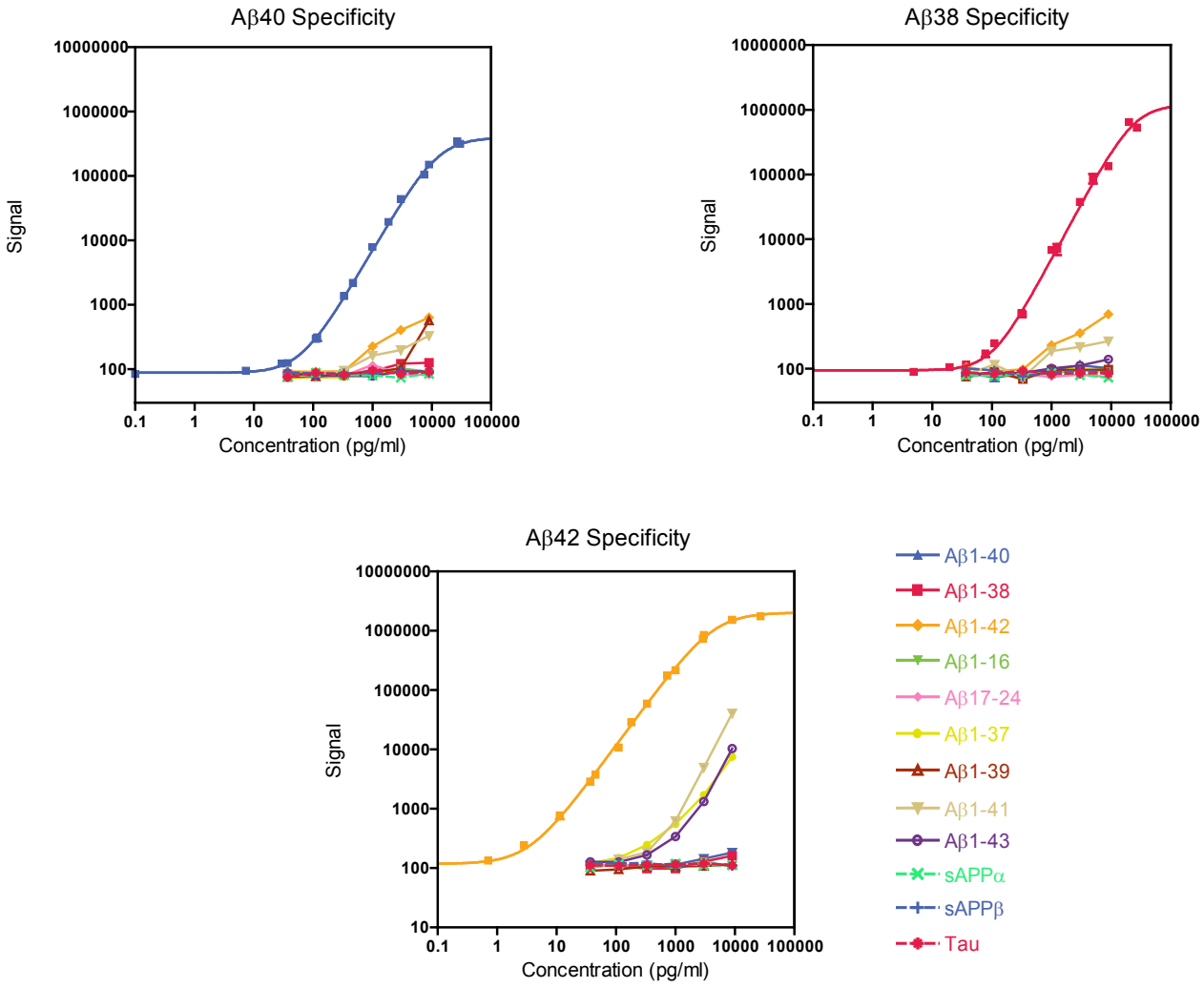
		Aβ40			Aβ38			Aβ42		
Kit Name	Sample Type	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
Aβ Peptide Panel 1 (4G8)	Human CSF (N=10)	88	11.6	70–114	96	5.2	90–112	92	11.0	79–125
	Mouse Plasma (N=10)	100	17.2	70–114	90	22.0	90–112	100	16.5	79–125
Aβ Peptide Panel 1 (6E10)	Human CSF (N=10)	95	10.9	73–131	96	6.1	85–113	95	10.7	80–132

Specificity

To assess specificity, each assay in the panel was tested individually. Cross-reactivity was less than 1.0% for all assays in the kit. Cross-reactivity was also evaluated with a panel of related Aβ peptides and other proteins of interest, including Aβ1-16, Aβ17-24, Aβ1-37, Aβ1-39, Aβ1-41, Aβ1-43, sAPPα, sAPPβ, and Tau. The Aβ42 assay exhibits minor cross-reactivity with some related Aβ peptides: Aβ1-41 (2.6%); Aβ1-43 and Aβ1-37 (<0.75%). Given the low endogenous levels of these peptides in CSF,[13,14] this is expected to have a minimal effect on Aβ42 quantification.

$$\%\ Nonspecificity = \frac{nonspecific\ signal}{specific\ signal} \times 100$$

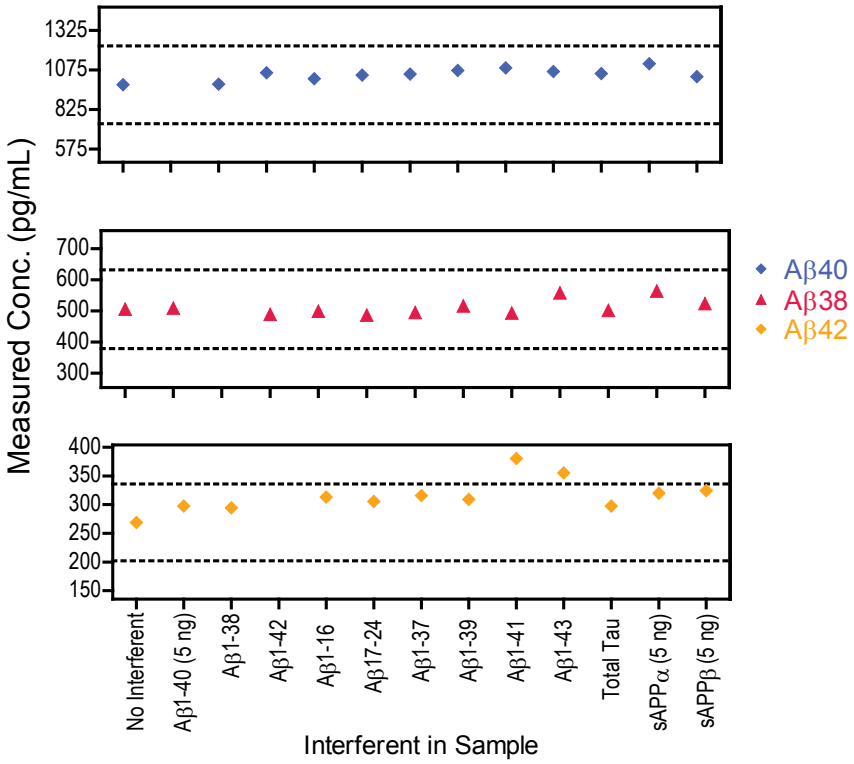
Figure 8. Aβ40, Aβ38, and Aβ42 assay specificity



Interference

Aβ40 (top), Aβ38 (middle), or Aβ42 (bottom) peptide calibrators were co-spiked into assay diluent with various Aβ peptides and proteins of interest at or above expected endogenous levels for these analytes (Aβ40, sAPPα, and sAPPβ spiked at 5 ng/mL; all other interferents spiked at 1 ng/mL). Measured Aβ levels were largely within 25% of the sample with no interferent, regardless of the spiked analyte or concentration.

Figure 9. Aβ40, Aβ38, and Aβ42 measured concentration with interferents



Stability

Assay kit calibrators and controls were tested for freeze–thaw stability. Results (not shown) demonstrated that calibrators and controls may go through 3 freeze/thaw cycles without significantly affecting the performance of the assay. In addition, thawed calibrators and controls are stable for ≥ 8 days when stored at 2–8°C or room temperature. 4G8 and 6E10 detection antibodies diluted for the assay are light stable for at least 4 hours. The kits are stable for 30 months from the date of manufacture. In addition, the validation study includes an on-going real-time stability study with scheduled performance evaluations of complete kits for up to 54 months from date of manufacture.

Tested Samples

Sample Range

Aβ peptides were measured in different sample types in order to determine the expected endogenous levels. Individual patient CSF samples were collected according to accepted protocols.[10] The commercial vendors that supplied the pooled CSF from remnant clinical samples were not able to adhere to stringent collection and handling procedures. CSF samples were diluted 2-fold prior to testing. Mouse plasma samples were diluted 4-fold prior to testing. Results for each sample set are reported below. Concentrations are corrected for sample dilution.

Table 11. Median and range of different sample types measured in the Aβ Peptide Panel 1 Kits

Kit Name	Sample Type	Statistic	Aβ40	Aβ38	Aβ42
Aβ Peptide Panel 1 (4G8)	Individual Patient Human CSF (N=23)	Median (pg/mL)	4,162	1,327	264
		Range (pg/mL)	2,112–7,458	689–2,910	37–706
		% Detected	100	100	100
	Pooled Remnant Human CSF (N=12)	Median (pg/mL)	2,324	670	195
		Range (pg/mL)	58.8–8,347	ND–3,340	3.96–748
		% Detected	100	92	100
	Mouse Plasma (N=20)	Median (pg/mL)	207	55.0	25.4
		Range (pg/mL)	113–317	ND–217	11.7–32.7
		% Detected	100	15	100
Aβ Peptide Panel 1 (6E10)	Individual Patient Human CSF (N=26)	Median (pg/mL)	3,831	1,293	187
		Range (pg/mL)	1,300–7,187	607–3,202	25–607
		% Detected	100	100	100
	Pooled Remnant Human CSF (N=12)	Median (pg/mL)	2,225	745	178
		Range (pg/mL)	43.7–7,012	ND–3,437	3.31–691
		% Detected	100	92	100

ND = Non-detectable

Disease Samples

Well-curated normal and individual AD patient CSF samples were diluted 2-fold prior to measuring with the Aβ Peptide Panel 1 (6E10) Kit. Samples handling was consistent with accepted protocols. The table below displays median and range of the measured concentrations for each sample set. Concentrations have been corrected for sample dilution. A graphical representation is also provided for the individual normal and AD patient samples. Comparable results were obtained with the 4G8 kit.

Figure 10. Normal and AD CSF samples measured in the Aβ Peptide Panel 1 (6E10) Kit

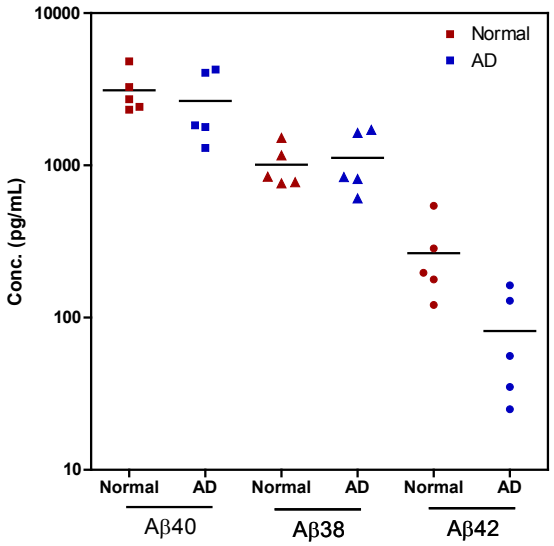


Table 12. Median and range of diseased samples measured in the Aβ Peptide Panel 1 (6E10) Kit

Kit Name	Sample Type	Statistic	Aβ40	Aβ38	Aβ42
Aβ Peptide Panel 1 (6E10)	Normal CSF (N=5)	Median (pg/mL)	2,715	842	197
		Range (pg/mL)	2,320–4,827	761–1,515	121– 542
		% Detected	100	100	100
	AD CSF (N=5)	Median (pg/mL)	1,831	837	56
		Range (pg/mL)	43.7–4,057	418–1,635	3.31–285
		% Detected	100	100	100

Assay Components

Calibrators

The assay calibrators are made using synthetic Aβ peptides.

Antibodies

Table 13. Antibody source species

		Source Species		
Kit Detection Antibody	Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
4G8	Aβ40	Mouse Monoclonal	Mouse Monoclonal	A
	Aβ38	Mouse Monoclonal	Mouse Monoclonal	A
	Aβ42	Mouse Monoclonal	Mouse Monoclonal	B
6E10	Aβ40	Mouse Monoclonal	Mouse Monoclonal	A
	Aβ38	Mouse Monoclonal	Mouse Monoclonal	A
	Aβ42	Mouse Monoclonal	Mouse Monoclonal	B

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Catalog Numbers

Table 14. Catalog numbers for the V-PLEX and V-PLEX Plus neurodegeneration biomarker multiplex and single assay kits*

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

*V-PLEX Plus kits include controls, plate seals, and wash buffer. See **Kit Components** for details.

Summary Protocol

Aβ Peptide Panel 1 Kits

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Aβ Peptide Panel 1 assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.
- ❑ Prepare calibrator solutions in Diluent 35 using the supplied calibrator.
- ❑ Dilute samples and controls in Diluent 35 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 100.
- ❑ Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

STEP 1: Add Diluent 35

- ❑ Add 150 µL/well of Diluent 35.
- ❑ Incubate at room temperature with shaking for 1 hour.

STEP 2: Wash and Add Detection Antibody Solution and Sample or Calibrator

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Add 25 µL/well of calibrator or diluted sample.
- ❑ Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Read Plate

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL/well of 2X Read Buffer T.
- ❑ Analyze the plate on the MSD instrument.

Plate Diagram