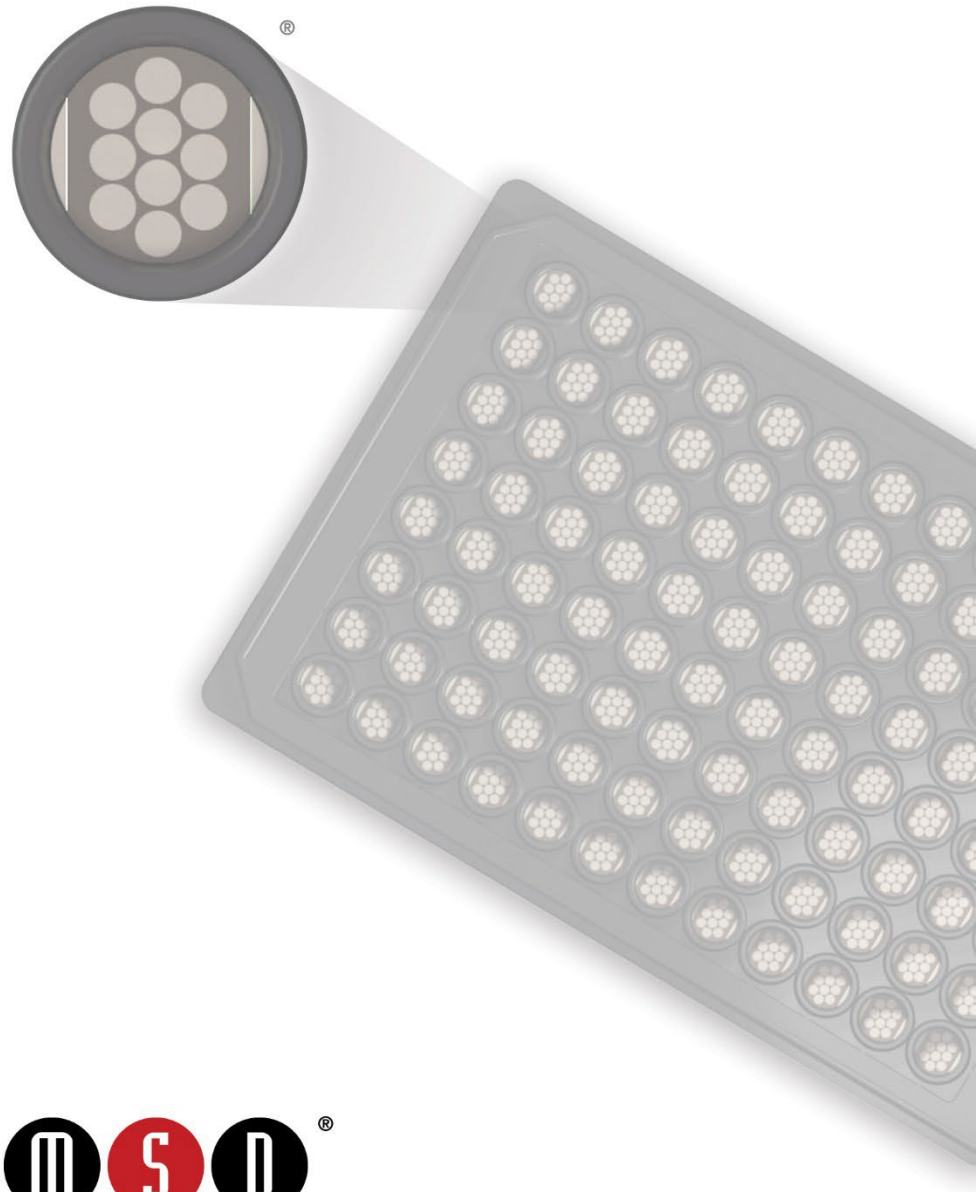
MSD® MULTI-SPOT Assay System

V-PLEX® Aβ Peptide Panel 2 Kits

Aβ38, Aβ40, Aβ42

V-PLEX®



SECTOR™ Assay Kits
QuickPlex Ultra™ Assay Kits

MSD®

MSD V-PLEX Platform

V-PLEX Aβ Peptide Panel 2 Kits

Aβ38, Aβ40, Aβ42

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

Instruments Supported:

SECTOR plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instruments

QuickPlex Ultra plates for use on MESO QuickPlex SQ 120, MESO QuickPlex SQ 120MM, and MESO QuickPlex Q 60MM instruments

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.
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Rockville, MD 20850 USA
www.mesoscale.com

The Aβ 4G8 and Human Aβ 6E10 antibodies used in MSD Aβ assays are supplied by BioLegend (formerly Covance Research Products).

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Contact Information

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Introduction

The MESO SCALE DISCOVERY® V-PLEX Aβ Peptide Panel 2 Kit measures beta-amyloid (Aβ) peptides derived from amyloid precursor protein (APP) including: Amyloid-beta 1-38 (Aβ38), Amyloid-beta 40 (Aβ40), and Amyloid-beta 42 (Aβ42). The markers of the V-PLEX Aβ Peptide Panel 2 enable the study of Alzheimer's disease (AD) and other neurodegenerative conditions. The V-PLEX Aβ Peptide Panel 2 Kits are available to study these peptides through both the 4G8 and 6E10 epitopes, enabling quantitative study of these important neurodegenerative markers in a variety of studies such as: Aβ aggregation, Amyloid cascade hypothesis, Aβ reduction through small molecule interactions, and other important studies.

Both Aβ Peptide Panels have been validated for human samples. The Ab Peptide Panel 2 (4G8) is also validated for mouse EDTA plasma.

Principle of the Assay

The V-PLEX Aβ Peptide Panel 2 Kit uses a sandwich immunoassay format on a MULTI-SPOT® 96-well plate, where individual capture antibodies are pre-coated directly onto spatially defined spots within each well (Figure 1).

During the assay, detection antibodies, samples, and calibrators are added to the plate simultaneously and allowed to incubate, forming sandwich complexes at each spot. After incubation and washing, MSD GOLD™ Read Buffer B is added to the plate. The plate containing the sandwich assay is loaded onto an MSD instrument. The instrument automatically performs the electrochemiluminescence (ECL) reaction by applying an electric current to each well. The SULFO-TAG™ label undergoes electrochemical excitation and emits light at ~620 nm, which is detected and quantified by the instrument for each individual spot. Signal is measured by an MSD instrument, which images each well and quantifies light emission from each individual spot.

Signal intensity is proportional to the amount of analyte captured at each spot. Quantitative analyte concentrations in unknown samples are calculated by interpolation from a calibration curve.

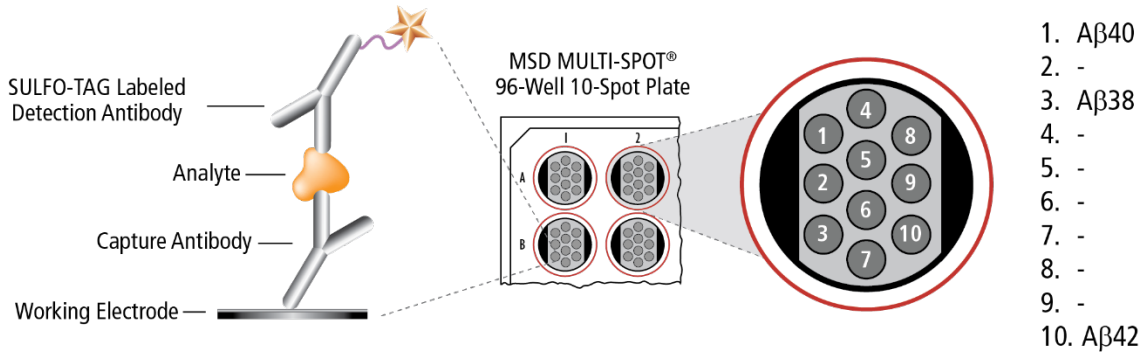

Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

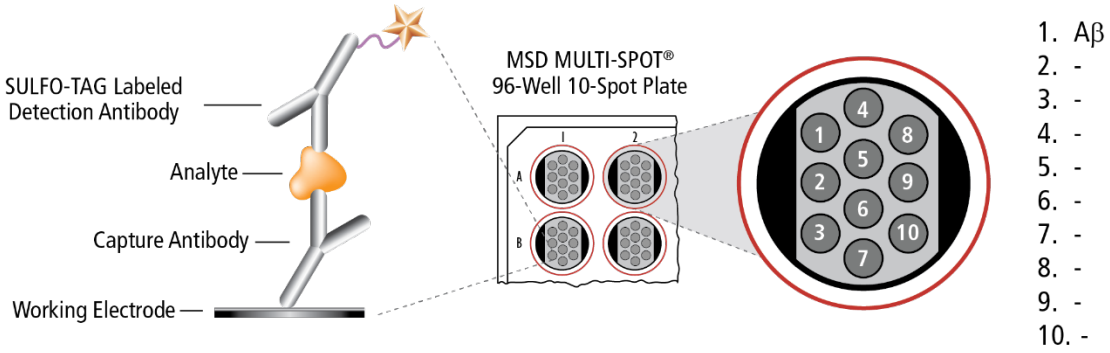

Figure 2. Small Spot plate diagram showing placement of the analyte capture antibody. Refer to Tables 1a and 1b for a list of assays provided on Small Spot plates.

Kit Components

V-PLEX Aβ Peptide Panel 2 assays are available as a multiplex kit, or as individual singleplex kits. V-PLEX Plus kits contain three control reagents in addition to the components in the V-PLEX kit. Kits include kit lot-specific (Table 1, Table 2) and non-kit lot-specific regents (Table 4, Table 6). Lot-specific information for each assay can be found in the certificate of analysis (COA).

See the **Catalog Numbers** section for complete kits.

Note: Components will be packaged by storage conditions for ease of storage and shipping.

Kit Lot-Specific Reagents and Components

Table 1a. SECTOR components that are supplied with the V-PLEX Aβ Peptide Panel 2 Kit.

Plates	Storage	Plate Type	Included Catalog # (-1, -2, -4)
Aβ Peptide Panel 2 (4G8) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K15764D/G, K150AYWD/G
Aβ Peptide Panel 2 (6E10) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K15765D/G, K150AYXD/G
Aβ40 Peptide (4G8) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K150AYSD/G
Aβ40 Peptide (6E10) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K150AYTD/G
Aβ42 Peptide (4G8) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K150AYUD/G
Aβ42 Peptide (6E10) 96-Well 10-Spot SECTOR Plate	2–8 °C	10-spot	K150AYVD/G

Catalog numbers ending in D represent V-PLEX kits, G represent V-PLEX Plus kits.

Table 1b. QuickPlex components that are supplied with the V-PLEX Aβ Peptide Panel 2 Kit.

Plates	Storage	Plate Type	Included Catalog # (-21, -22, -24)
Aβ Peptide Panel 2 (4G8) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K15764D/G, K150AYWD/G
Aβ Peptide Panel 2 (6E10) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K15765D/G, K150AYXD/G
Aβ40 Peptide (4G8) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K150AYSD/G
Aβ40 Peptide (6E10) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K150AYTD/G
Aβ42 Peptide (4G8) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K150AYUD/G
Aβ42 Peptide (6E10) 96-Well 10-Spot QuickPlex Ultra Plate	2–8 °C	10-spot	K150AYVD/G

Catalog numbers ending in D represent V-PLEX kits, G represent V-PLEX Plus kits.

Table 1c. Calibrator and diluent that are supplied with the V-PLEX Aβ Peptide Panel 2 Kit.

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Aβ1-38 Peptide (4G8)	≤-70 °C	C002Y-2	30 µL	1 vial	5 vials	25 vials	Synthetic peptide calibrator
Aβ1-40 Peptide (4G8)	≤-70 °C	C002Z-2	30 µL	1 vial	5 vials	25 vials	
Aβ1-42 Peptide (4G8)	≤-70 °C	C003A-2	30 µL	1 vial	5 vials	25 vials	
Aβ1-38 Peptide (6E10)	≤-70 °C	C00NZ-2	30 µL	1 vial	5 vials	25 vials	
Aβ1-40 Peptide (6E10)	≤-70 °C	C000A-2	30 µL	1 vial	5 vials	25 vials	
Aβ1-42 Peptide (6E10)	≤-70 °C	C01LB-2	30 µL	1 vial	5 vials	25 vials	
Diluent 35	2–8 °C	R50AE-3	30 mL	1 bottle	—	—	Diluent for samples and calibrator
		R50AE-2	150 mL	—	1 bottle	5 bottles	
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Diluent for detection antibody

Table 2. Individual detection antibodies for each assay supplied with specific kits.

SULFO-TAG Conjugated Detection Antibody	Storage	Cap color	Catalog #	Size	Quantity Supplied		
					1 Plate	5 Plates	25 Plates
Aβ 4G8 Antibody	2–8 °C	●	D20AYC-2	75 µL	1	—	—
		●	D20AYC-3	375 µL	—	1	5
Human Aβ 6E10 Antibody	2–8 °C	●	D21AYD-2	75 µL	1	—	—
		●	D21AYD-3	375 µL	—	1	5

Dash (—) = not applicable.

Table 3. Antibody source.

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

Non-Kit Lot-Specific Reagents and Components

Table 4. Reagents that are supplied with V-PLEX and V-PLEX Plus Aβ Peptide Panel 2 Kits.

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Aβ40 Blocker*	RT	R93BJ-1	40 µL	1	—	—	Blocking reagent for CSF samples only
		R93BJ-2	200 µL	—	1	5	
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent reaction
		R60AM-2	90 mL	—	1 bottle	5 bottles	

*Kits that include the Aβ40 assay are supplied with Aβ40 Blocker

RT = room temperature.

Dash (—) = not applicable.

Table 5. Synthetic peptides are used in the calibrators.

	Expression System
Aβ1-38 4G8	*Synthetic Peptide*
Aβ1-40 4G8	*Synthetic Peptide*
Aβ1-42 4G8	*Synthetic Peptide*
Aβ1-38 6E10	*Synthetic Peptide*
Aβ1-40 6E10	*Synthetic Peptide*
Aβ1-42 6E10	*Synthetic Peptide*

Additional Components

Controls are provided as components in the Neurodegeneration Control Pack 1 (4G8) (Catalog No. C40RQ-1) and in the Neurodegeneration Control Pack 1 (6E10) (Catalog No. C41LB-1). These Control Packs consist of 3 levels of controls, each containing concentrations of synthetic peptides that are detected by the V-PLEX and V-PLEX Plus Aβ Peptide Panel 2 Kits. The controls span the linear range and are designed to test the analytical range of the assays. These controls assess reproducibility of assay performance, and precision CVs are expected to be less than 25%. The certificate of analysis contains the concentrations of the controls measured at MSD across three lots. Even with good laboratory practices, site-to-site differences may occur; therefore, to establish accuracy specifications, it is recommended that each lab establish its own nominal values and acceptance range for the control concentrations.

Table 6. Additional components that are supplied with V-PLEX Plus Aβ Peptide Panel 2 Kits.

Reagent	Storage	Cap Color	Catalog #	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
Neurodegeneration Control 1 (4G8)	≤-70 °C		C40RQ-1	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls
Neurodegeneration Control 2 (4G8)	≤-70 °C			1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 3 (4G8)	≤-70 °C			1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 1 (6E10)	≤-70 °C		C41LB-1	1 vial	1 vial	5 vials	25 vials	Multi-analyte controls
Neurodegeneration Control 2 (6E10)	≤-70 °C			1 vial	1 vial	5 vials	25 vials	
Neurodegeneration Control 3 (6E10)	≤-70 °C			1 vial	1 vial	5 vials	25 vials	
Wash Buffer	RT	—	R61AA-1	100 mL	1 bottle	1 bottle	5 bottles	20-fold concentrated plate wash buffer solution
Plate Seals	—	—	—	—	3	15	75	Adhesive seals for sealing plates during incubations

Dash (—) = not applicable.

Additional Materials and Equipment

Materials

- ❑ 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
- ❑ MSD Wash Buffer catalog no. R61AA-1 (included in V-PLEX Plus kits)
- ❑ Adhesive plate seals (3 per plate included in V-PLEX Plus kits)
- ❑ Deionized water
- ❑ OPTIONAL: Neurodegeneration Control Pack 1, available for separate purchase from MSD, catalog no. C40RQ-1 (4G8) or C41LB-1 (6E10) (included in V-PLEX Plus kits)

Equipment

- ❑ Plate-washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Vortex mixer
- ❑ Water bath
- ❑ OPTIONAL: Centrifuge for sample preparation

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay 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), which can be obtained from MSD Customer Service or at the www.mesoscale.com® website.

Best Practices

Read this product insert before use, and follow the best practices:

Reagent Preparation

Do Not Mix Lots	Do not mix components between boxes of multiplex V-PLEX panels. Lot information is provided in the lot-specific COA.
Thaw Reagents	Bring frozen diluents to room temperature in a 22–25 °C water bath. Thaw other reagents on wet ice and use them immediately. Mix well before use. Bring plates to room temperature before opening the packet.

Reagent Handling

Avoid Bubbles During Pipetting	Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is especially important when adding read buffer (just prior to reading the plate).
Prepare in Polypropylene Microcentrifuge Tubes	Prepare standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution, and mix by vortexing after each dilution.
Avoid Prolonged Light Exposure	Avoid prolonged exposure of the detection reagent (stock or diluted) to light. During the plate incubation steps, plates do not need to be shielded from light (except for direct sunlight).

Plate Handling

Plate Shaking Guidelines	Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm for 96-well plates. Keep the shaking speed and shaker model consistent for long-term studies. Binding reactions may reach equilibrium sooner if shaken in the middle of this range (~700 rpm) or above.
Washing Fluid Removal	Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
Some Assays Are Temperature Sensitive	V-PLEX assays were characterized at 20–26 °C. Assays run above or below that range may be negatively affected.

Plate Reading

Remove Plate Seal	Remove the plate seal before reading the plate in the instrument.
Do Not Shake Plate	Do not shake the plate after adding read buffer.
Room Temperature Read Buffer	Read buffer should be at room temperature (20-26 ºC) when adding it to the plate. Do NOT shake the read buffer bottle before use.
Timing of Plate Reads	Keep time intervals consistent between the addition of read buffer and reading the plate to improve interplate precision. Prepare an MSD instrument before adding read buffer.
Results Above Curve	If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Summary Protocol: Multiplex, Singleplex Aβ38 & Aβ40

Aβ Peptide Panel 2 Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol before performing the Aβ Peptide Panel 2 assays.

Step 1: Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.
- ❑ Prepare calibration solutions in Diluent 35 using the supplied calibrator.
  - Thaw the frozen calibrator blend, then dilute in Diluent 35.
  - Vortex briefly using short pulses.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- ❑ Dilute samples and controls 2-fold in Diluent 35 before adding to the plate. Thaw controls before dilution.
- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 100.

STEP 2: Add Blocker

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

STEP 3: Wash and Add Detection Antibody Solution and Sample

- ❑ Wash plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 25 µL/well of detection antibody solution and 25 µL/well of sample (calibrators, controls, or unknowns) to each well.
- ❑ Incubate at room temperature with shaking for 2 hours.

STEP 4: Wash and Read Plate

- ❑ Wash plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL/well of MSD GOLD Read Buffer B.
- ❑ Analyze plate on the MSD instrument.

Summary Protocol: Singleplex Aβ42

Aβ42 Peptide Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol before performing the singleplex Aβ42 Peptide assay.

Step 1: Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.
- ❑ Prepare calibration solutions in Diluent 35 using the supplied calibrator.
  - Thaw the frozen calibrator blend, then dilute in Diluent 35.
  - Vortex briefly using short pulses.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator.
- ❑ Dilute samples and controls 2-fold in Diluent 35 before adding to the plate. Thaw controls before dilution.
- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 35.

STEP 2: Add Blocker

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

STEP 3: Wash and Add Sample, Control, or Calibrator

- ❑ Wash plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 50 µL/well of calibrator, diluted sample, or diluted control to each well.
- ❑ Incubate at room temperature with shaking for 2 hours.

STEP 4: Wash and Add Detection Antibody Solution

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

STEP 5: Wash and Read Plate

- ❑ Wash plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL/well of MSD GOLD Read Buffer B.
- ❑ Analyze plate on the MSD instrument.

Recommended Protocol

Bring all reagents to room temperature and refer to the **Best Practices** section (above) before beginning the protocol.

Important: Upon the first thaw, aliquot Diluent 35 into suitable volumes before refreezing.

Reagents prepared at each step are sufficient for a one-plate experiment.

STEP 1: PREPARE SAMPLES AND REAGENTS

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution. Dilute the stock solution to 1X before use.

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

Prepare Calibrator Dilutions

MSD supplies individual Aβ peptide calibrator that yields the recommended highest calibrator concentration when diluted 40-fold in Diluent 35. MSD supplies the Aβ42 singleplex kit calibrator that yields the recommended highest calibrator concentration when diluted 20-fold in Diluent 35.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates (Figure 3):

- ❑ Prepare the most concentrated calibrator (Calibrator 1) by adding 10 µL of thawed stock calibrator(s) with the appropriate volume of Diluent 35. Mix well by vortexing.

  Instructions for Aβ Peptide Panel 2 multiplex kits (Figure 3):

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

  Instructions for Aβ singleplex kits (Figure 4):

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

  Instructions for Aβ42 singleplex kits:

  Add 20 µL of the supplied peptide calibrator to 380 µL of Diluent 35.
- ❑ Prepare the next calibrator by transferring 100 µL of Calibrator 1 to 300 µL of Diluent 35. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
- ❑ Use Diluent 35 as the zero calibrator.

Note: Stock (40X) calibrator can be stored at 4ºC for up to 7 days. It may also be stored at ≤70ºC and is stable through five freeze thaw cycles. Diluted (1X) calibrator can be stored at 4ºC for up to 1 day. Do not store calibrator at RT beyond the day of testing. For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.

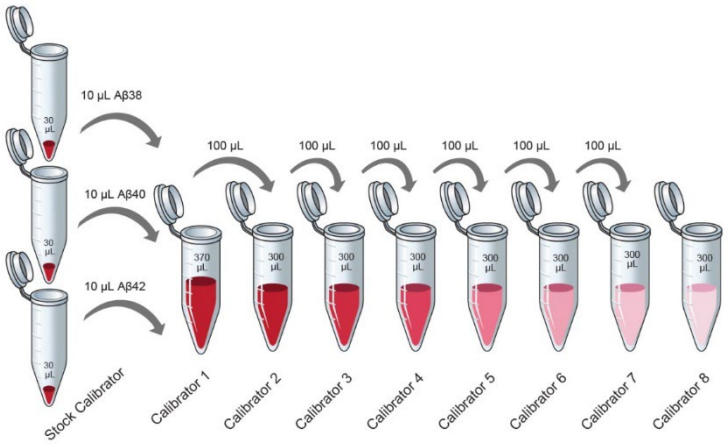

Figure 3. Dilution schema for preparation of Calibrator Standards for Aβ Peptide Panel 2 multiplex kit.

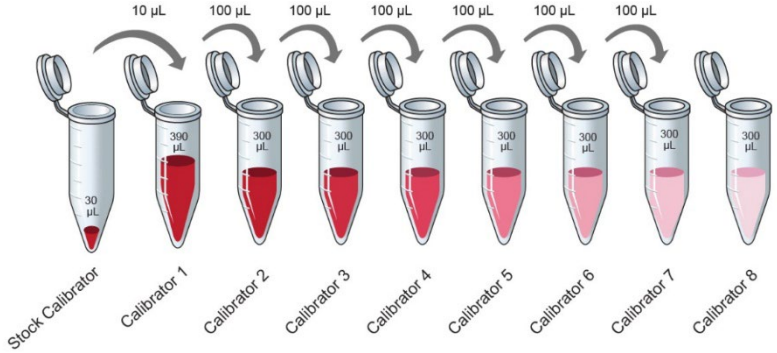

Figure 4. Dilution schema for preparation of Calibrator Standards for Aβ singleplex assay kit.

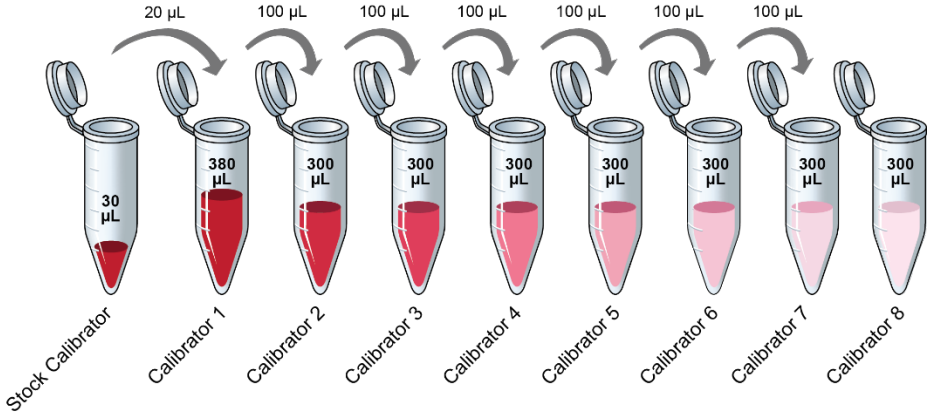

Figure 5. Dilution schema for preparation of Calibrator Standards for the Aβ42 singleplex assay kit.

Dilute Samples

Dilute samples with Diluent 35. For human CSF MSD recommends a minimum 2-fold dilution. For example, when running samples in duplicate, add 60 µL of sample to 60 µL of Diluent 35. We recommend running at least two replicates per sample. You may conserve sample volume by using a higher dilution. For mouse plasma samples MSD recommends a minimum 4-fold dilution (Aβ Peptide Panel 2 (4G8) Kit only). For example, when running samples in duplicate, add 30 µL of sample to 90 µL of Diluent 35.

For the Aβ42 singleplex assay MSD recommends a minimum 8-fold dilution with Diluent 35. For example, when running samples in duplicate, add 15 µL of sample to 105 µL of Diluent 35. We recommend running at least two replicates per sample.

All kits include diluent sufficient enough for running samples in duplicate. Additional diluent can be purchased at www.mesoscale.com.

Prepare Controls

Three levels of multi-analyte controls are available for separate purchase from MSD in the Neurodegeneration Control Pack 1, catalog no. C40RQ-1 (4G8) or C41LB-1 (6E10). (Controls are included only in V-PLEX Plus kits.)

Instructions for Aβ Peptide Panel 2 multiplex kits and Aβ38, Aβ40 singleplex kits:

Thaw the controls on wet ice for at least 30 minutes. Dilute controls 2-fold in Diluent 35. Mix by vortexing.

Instructions for Aβ42 singleplex kits:

Thaw the controls on wet ice for at least 30 minutes. Dilute controls 8-fold in Diluent 35. Mix by vortexing.

Note: Stock (2X) controls are provided frozen and must be stored frozen and are stable through 5X freeze-thaw cycles. Stability of diluted (1X) controls has not been tested. For the lot-specific concentration of each analyte in the control, refer to the COA supplied with the control pack.

Prepare Detection Antibody Solution

MSD provides each detection antibody separately as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use.
For kits that include the Aβ40 assay, the Aβ40 detection antibody 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 detection antibodies and add to 2,910 µL of Diluent 100:

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

Detection Antibody Solution without Aβ40 Blocker

For one plate, combine the following detection antibodies and add to 2,940 µL of Diluent 100:

- ❑ 60 µL of 50X SULFO-TAG Anti-Aβ 4G8 Antibody or Anti-Aβ 6E10 Antibody

STEP 2: Add Blocker

MSD V-PLEX 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. Prewash plates before use as recommended in the assay protocol.

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ 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.

Note: Washing the plate before sample addition is an optional but recommended step that may provide greater uniformity of results for certain assays. Analytical parameters, including limits of quantification, recovery of controls, and sample quantification, are not affected by washing the plate before sample addition.

REMAINING STEPS: Multiplex, Singleplex Aβ38 & Aβ40 Kits

STEP 3: Wash and Add Detection Antibody Solution and Sample

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

STEP 4: Wash and Read Plate

Wash and Add MSD GOLD Read Buffer B

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

Note: MSD GOLD Read Buffer B is provided at the working concentration for the assay. Diluting MSD GOLD Read Buffer B may compromise assay results.

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well.
- ❑ Analyze the plate on an MSD instrument.

REMAINING STEPS: Singleplex Aβ42 Kits

STEP 3: Wash and Add Sample, Control, or Calibrator

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 50 µL/well of calibrator, diluted sample, or diluted control to each well.
- ❑ Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

STEP 4: Wash and Add Detection Antibody Solution

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

STEP 5: Wash and Read Plate

Wash and Add MSD GOLD Read Buffer B

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

Note: MSD GOLD Read Buffer B is provided at the working concentration for the assay. Diluting MSD GOLD Read Buffer B may compromise assay results.

- ❑ Wash the plate 3 times with at least 150 µL/well of Wash Buffer.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well.
- ❑ Analyze the plate on an MSD instrument.

Catalog Numbers

Table 7. Catalog numbers for V-PLEX and V-PLEX Plus Aβ Peptide Panel 2 (human) multiplex and individual assays.

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 2 (4G8)	K15764D-1	K15764D-2	K15764D-4	K15764G-1	K15764G-2	K15764G-4
Aβ Peptide Panel 2 (4G8) QuickPlex	K15764D-21	K15764D-22	K15764D-24	K15764G-21	K15764G-22	K15764G-24
Aβ Peptide Panel 2 (6E10)	K15765D-1	K15765D-2	K15765D-4	K15765G-1	K15765G-2	K15765G-4
Aβ Peptide Panel 2 (6E10) QuickPlex	K15765D-21	K15765D-22	K15765D-24	K15765G-21	K15765G-22	K15765G-24
Individual SECTOR Assay Kits						
Aβ38 Peptide (4G8)	K150AYWD-1	K150AYWD-2	K150AYWD-4	K150AYWG-1	K150AYWG-2	K150AYWG-4
Aβ38 Peptide (6E10)	K150AYXD-1	K150AYXD-2	K150AYXD-4	K150AYXG-1	K150AYXG-2	K150AYXG-4
Aβ40 Peptide (4G8)	K150AYSD-1	K150AYSD-2	K150AYSD-4	K150AYSG-1	K150AYSG-2	K150AYSG-4
Aβ40 Peptide (6E10)	K150AYTD-1	K150AYTD-2	K150AYTD-4	K150AYTG-1	K150AYTG-2	K150AYTG-4
Aβ42 Peptide (4G8)	K150AYUD-1	K150AYUD-2	K150AYUD-4	K150AYUG-1	K150AYUG-2	K150AYUG-4
Aβ42 Peptide (6E10)	K150AYVD-1	K150AYVD-2	K150AYVD-4	K150AYVG-1	K150AYVG-2	K150AYVG-4
Individual QuickPlex Ultra Assay Kits						
Aβ38 Peptide (4G8)	K150AYWD-21	K150AYWD-22	K150AYWD-24	K150AYWG-21	K150AYWG-22	K150AYWG-24
Aβ38 Peptide (6E10)	K150AYXD-21	K150AYXD-22	K150AYXD-24	K150AYXG-21	K150AYXG-22	K150AYXG-24
Aβ40 Peptide (4G8)	K150AYSD-21	K150AYSD-22	K150AYSD-24	K150AYSG-21	K150AYSG-22	K150AYSG-24
Aβ40 Peptide (6E10)	K150AYTD-21	K150AYTD-22	K150AYTD-24	K150AYTG-21	K150AYTG-22	K150AYTG-24
Aβ42 Peptide (4G8)	K150AYUD-21	K150AYUD-22	K150AYUD-24	K150AYUG-21	K150AYUG-22	K150AYUG-24
Aβ42 Peptide (6E10)	K150AYVD-21	K150AYVD-22	K150AYVD-24	K150AYVG-21	K150AYVG-22	K150AYVG-24