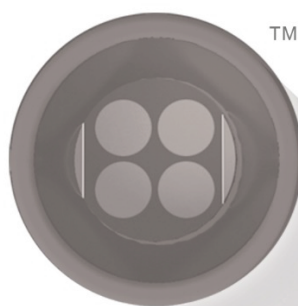


MSD[®] MULTI-SPOT Assay System

Human A β 42 Kit

V-PLEX[®]



	V-PLEX[®]	V-PLEX Plus
1-Plate Kit	K151LBE-1	K151LBG-1
5-Plate Kit	K151LBE-2	K151LBG-2
25-Plate Kit	K151LBE-4	K151LBG-4



MSD Neurodegenerative Disease Assays

Human A β 42 Kit

For use with cerebrospinal fluid (CSF), cell lysates, conditioned cell culture media, 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.

MESO SCALE DISCOVERY[®]

A division of Meso Scale Diagnostics, LLC.

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Introduction

The A β 42 peptide is a C-terminal cleavage product of amyloid precursor protein (APP), a neuronal transmembrane protein. Under normal circumstances, A β 42 may play a role in cellular homeostasis. In a variety of neurodegenerative diseases, A β 42 accumulates and aggregates, resulting in significant physical and functional changes to the brain. Most notably, A β 42 is the primary component of the neuritic plaques that are characteristic of Alzheimer's disease (AD) and, together with tau, has emerged as a core biomarker of the disease. Tau and A β 42 levels in CSF are effective in discriminating incipient AD from age-related memory impairment, depression, and some secondary dementias.¹⁻³ Studies aimed at evaluating the association between AD-type pathologic changes in the brain and antemortem CSF levels of A β 42 and tau protein indicated that levels of both proteins correlated with the presence of neurofibrillary tangles and A β in the brain.⁴

The MSD A β 42 Assay has been validated for the detection of A β 42 in CSF. The kit may also be used to measure A β 42 levels in cell lysates, conditioned media prepared from cultured human neuronal cells, and human tissue homogenates. Standardized assays that have minimal variability across manufacturing runs, users, and platforms are needed for accurate analysis of AD markers.⁵ MSD is committed to providing state-of-the-art biomarker measurements to the neurodegenerative disease research community and has independently been engaged in the identification and elimination of potential causes of assay variability. Through the use of highly characterized critical reagents and improved handling methods, the Human A β 42 Kit provides a new level of robustness and reliability.

Principle of the Assay

MSD neurodegenerative disease assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human A β 42 is a sandwich immunoassay. MSD provides a plate that has been precoated with a capture antibody for total A β 42. The user adds the sample and a solution containing the detection antibody conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) throughout one or more incubation periods. Analyte in the sample binds to the capture antibody immobilized on the working electrode surface; recruitment of the conjugated detection antibody by bound analytes completes the sandwich. The user adds an MSD read 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 intensity of emitted light to provide a quantitative measure of analyte in the samples. 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.⁶

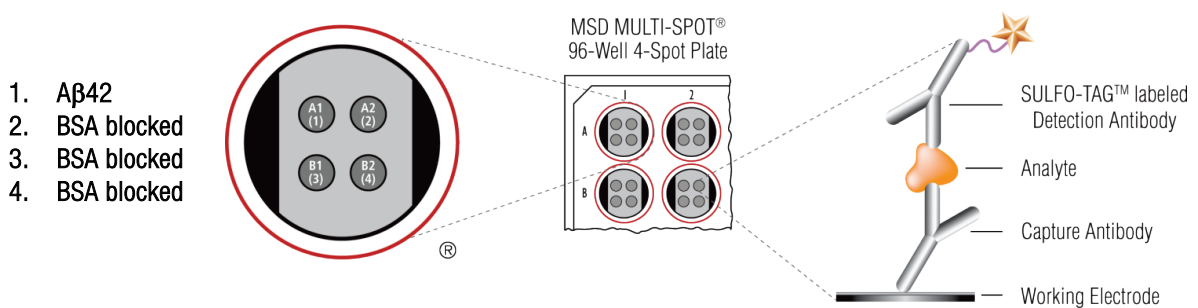


Figure 1. Spot diagram showing the placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Kit Components

The Human A β 42 assay is available in V-PLEX and V-PLEX Plus kits. V-PLEX Plus kits include additional items (controls, wash buffer, and plate seals). See below for details.

Reagents Supplied With All Kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
MULTI-SPOT® 96-Well Human A β 42 Plate	2–8 °C	N451LBA-1	4-Spot	1 plate	5 plates	25 plates	96-well plate, foil-sealed, with desiccant
A β 42 Peptide (6E10) (20X)	≤-70 °C	C01LB-2	1 vial	1 vial	5 vials	25 vials	Synthetic peptide calibrator in diluent that mimics human CSF. Analyte concentration is provided in the lot-specific certificate of analysis (COA).
SULFO-TAG Anti-A β 6E10 Antibody (50X) ¹	2–8 °C	D21LB-2	75 μ L	1 vial	-	-	SULFO-TAG conjugated antibody
		D21LB-3	375 μ L	-	1 vial	5 vials	
Diluent 35	2–8 °C	R50AE-3	30 mL	1 bottle	-	-	Diluent for samples, calibrator, and detection antibody; contains proteins and preservatives.
		R50AE-2	150 mL	-	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.

RT = room temperature
Dash (-) = not applicable

V-PLEX Plus Kits: Additional Components

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate KI	
Neurodegeneration Control 1 (6E10)*	≤-70 °C	C41LB-1	100 μ L	1 vial	5 vials	25 vials	Multi-analyte controls in diluent that mimics human CSF. The concentration of the controls is provided in the lot-specific certificate of analysis.
Neurodegeneration Control 2 (6E10)*	≤-70 °C	C41LB-1	100 μ L	1 vial	5 vials	25 vials	
Neurodegeneration Control 3 (6E10)*	≤-70 °C	C41LB-1	100 μ L	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 in the Neurodegeneration Control Pack 1 (catalog No. C41LB-1)
Dash (-) = not applicable

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 the 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
- Phosphate-buffered saline (PBS) plus 0.05% Tween-20 for plate washing or MSD Wash Buffer, catalog No. 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, available for separate purchase from MSD, catalog No. C41LB-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 materials properly in accordance with local, state, and federal guidelines. Additional product-specific safety information is available in the 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. This may compromise assay performance. Lot information is provided in the lot-specific COA.
- To get the most consistent results between runs, keep “room temperature” for incubations between 20 and 26 °C.
- Bring diluent(s) to room temperature in a 24 °C water bath. Thaw other reagents as directed in the Reagent Preparation section.
- 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 when necessary to avoid the introduction of bubbles, and for empty wells, pipette to the bottom corner.
- Plate 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.
- 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 the sample or detection antibody solution in the plate.
- Remove plate seals before reading the plate.
- If assay results are above the top of the calibration curve, dilute 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 30 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 and samples should be prepared during the blocking step and used within one hour of preparation.

Prepare Calibrator Dilutions

MSD recommends using a 7-point calibration curve for the Human A β 42 Assay. All samples tested during assay validation were contained within the range of the calibration curve. In certain sample populations, it may be advantageous to add an additional higher calibrator. The intermediate dilution in Step 2 below can be used as the highest calibrator for an optional calibration curve as shown in the typical calibration curve section on page 11.

To prepare 7 calibrator solutions plus a zero calibrator for up to 3 replicates:

- 1) Vortex the thawed calibrator stock vial.
- 2) Prepare an intermediate dilution by adding 20 μ L of the 20X calibrator stock to 380 μ L of Diluent 35. Mix well by vortexing.
- 3) Prepare the highest calibrator by transferring 100 μ L of the diluted calibrator to 300 μ L of Diluent 35. Mix well by vortexing. Repeat 4-fold serial dilutions 6 additional times to generate 7 calibrators.
- 4) Use Diluent 35 as the zero calibrator.

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

Sample Collection and Handling

Sample collection methods and pre-analytical conditions may cause variability in measured A β 42 levels. MSD recommends reviewing current literature and protocols such as those proposed by the Alzheimer's Disease Neuroimaging Initiative (ADNI).7-9 The samples described below were clarified through a single centrifugation step at 1200 relative centrifugal force for 10 minutes at 2–8°C. Samples were then aliquoted and frozen to minimize potential freeze/thaw effects.

Human CSF samples should be diluted to minimize matrix effects. We recommend diluting samples 8-fold in Diluent 35; however, you may need to use higher or lower dilution factors depending on the sample set under investigation. CSF samples measured using the Human A β 42 Kit exhibit good linearity; please see the Dilution Linearity section for representative data.

- 1) Vortex the thawed sample.
- 2) Dilute 8-fold in Diluent 35.
- 3) Vortex the diluted sample before adding to the MSD plate.

Prepare Controls

Three levels of multi-analyte frozen liquid controls are available for purchase from MSD in the Neurodegeneration Control Pack, catalog No. C41LB-1. (Controls are included in V-PLEX Plus Kits.) The controls are prepared by spiking known levels of synthetic A β 42 peptide into a diluent that mimics human CSF.

Thaw controls at room temperature and mix well by vortexing. Dilute controls 8-fold in Diluent 35 and mix well by vortexing.

For lot-specific concentrations of controls, refer to the COA supplied with the product.

Prepare Detection Antibody Solution

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

For one plate, combine:

- 60 μ L of 50X SULFO-TAG Anti-A β (6E10) Antibody
- 2.94 mL of Diluent 35

Prepare Wash Buffer

MSD recommends using Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog No. R61AA-1 (included in V-PLEX Plus kit). MSD Wash Buffer is provided as a 20X stock solution. The working solution is 1X.

For 1 plate, combine:

- 15 mL of wash buffer (20X)
- 285 mL of deionized water

Prepare Read Buffer

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

For one plate, combine:

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

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

The plates have been coated with antibody for the analyte as shown in 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

Dilute calibrator, samples, and controls during the step 1 incubation.

1. **Add Diluent 35:** Dispense 150 μL of Diluent 35 into each well of the MSD plate. Seal the plate with an adhesive plate seal, and incubate at room temperature with shaking for 1 hour.
2. **Wash and Add Sample, Control, or Calibrator:** Wash the plate 3 times with at least 150 μL /well of PBS-T. Add 50 μL of calibrator, diluted sample, or diluted control per well. Seal the plate with an adhesive plate seal, and incubate at room temperature with shaking for 1 hour.
 - a. You may prepare detection antibody solution during incubation.
3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with at least 150 μL /well of PBS-T. Add 25 μL of 1X detection antibody solution to each well of the MSD plate. Seal the plate with an adhesive plate seal, and at room temperature with shaking for 1 hour.
 - a. You may prepare diluted read buffer during incubation.
4. **Wash and Read:** Wash the plate 3 times with at least 150 μL /well of PBS-T. Add 150 μL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Validation

The Human A β 42 Kit was validated for the detection of A β 42 peptide in human CSF. The kit meets the levels of consistency and robustness outlined in “Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement” by J. W. Lee, et al.⁶ The assay was validated using three independently-built kit lots tested by multiple analysts across multiple runs and days. Each kit lot was built using different lots of raw materials. Human CSF-based validation samples with A β 42 concentrations that spanned the calibration curve were built and used to validate the dynamic range of the assay.

Details of the assay validation procedure are described in the sections below. Briefly, kit calibration curve, limit of quantification samples (calibrator spiked into diluent), and matrix-based validation samples and controls were measured across multiple kit lots, days, plates, and analysts to assess kit sensitivity, accuracy, and precision. Spike recovery and dilution linearity were assessed using individual normal and Alzheimer’s disease patient samples. Assay specificity and interference were evaluated using a panel of related peptides and proteins. Assay tolerance to sample contamination with hemolyzed blood was also evaluated. Assay robustness and stability were assessed through freeze-thaw testing and accelerated stability studies (calibrators, antibodies, controls); these studies were augmented with real-time stability studies on complete kits conducted up to 18 months after the date of manufacture. Specifications for and representative data from these tests are described in the sections below.

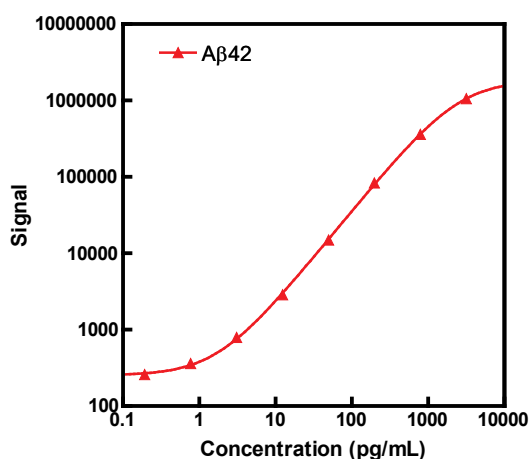
Analysis of Results

The calibration curve is modeled using least squares fitting algorithms to calculate the concentration of an analyte in the samples using signals from the calibrators. The assays have a wide dynamic range (4 logs), which 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.

The 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

Calibration curve accuracy and precision were assessed for 3 kit lots. Representative calibration curve data from one kit lot are presented below. The data were collected over six days of testing by three analysts (23 runs in total). In-well concentrations are reported in the table. An 8-point curve was used to validate the assay over a broad dynamic range.



Conc. (pg/mL)	Aβ42 ²	
	Average Signal	%CV
0	245	5.8
0.193	258	4.0
0.774	361	2.6
3.10	793	2.9
12.4	2870	4.3
49.5	14 931	4.1
198	83 291	3.2
793	359 727	3.3
3170	1 054 859	3.7

Calibrators that fall between the assay's upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) have a low intra-plate calculated concentration coefficient of variance (%CV, <20%) and good recovery (80–120%).

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

Sensitivity

Assay sensitivity and dynamic range were assessed by testing across multiple kit lots, analysts, and runs. The lower limit of detection (LLOD) and upper and lower limits of quantification (ULOQ and LLOQ, respectively) for each of three independent kit lots were determined. Testing for each kit involved a minimum of 12 runs conducted by three analysts across at least 3 days of testing (N=42 runs across three kit lots). A summary of the Human A β 42 Assay sensitivity and dynamic range is presented in the table below. In-well concentrations are reported.

The lower limit of detection (LLOD) is a calculated concentration based on a signal of 2.5 standard deviations above the blank (zero calibrator). The range of LLODs measured across three kit lots (N=66 plates) is presented.

The ULOQ and LLOQ were determined by spiking a known value of calibrator into diluent to assess the accuracy and precision of the samples.

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

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 for download at www.mesoscale.com.

	A β 42 (μ g/mL)
LLOD Range	0.070–0.96
LLOQ	3.0
ULOQ	2000

Precision

Control samples using pooled human CSF with or without spiked A β 42 calibrator were built. Two sets of control samples were independently prepared and tested with the Human A β 42 Kit. Each set contained three controls with A β 42 levels spanning the expected range of A β 42 in human CSF samples. Controls were diluted 8-fold. Concentrations for all controls were measured using three independent Human A β 42 Kit lots. Representative data from one set of controls is presented in the tables below. For this study, three analysts ran tests over 11 days (N=29 runs across three kit lots). The control data for each kit lot and an inter-kit lot summary are presented in the upper table. Concentrations presented in the table have been dilution-adjusted. Average intraplate Calc. Conc. %CV is the average concentration CV of the control replicates on an individual plate. Interplate Calc. Conc. %CV is the variability of measured control concentrations across plates, with replicate information as indicated in the table. Total error was calculated as (interplate Calc. Conc. %CV) + (absolute value of % Conc. Recovery Relative to Final Expected Concentration–100%). The concentrations presented in the inter-lot summary represent the expected concentration for each control. Measured concentrations for each kit lot relative to the final expected concentrations are presented in the lower table.

The controls had low variability (CVs <20%), and the control concentrations measured in each kit lot were within 10% of the expected value (lower table).

We verify assay precision for each new lot; results are provided in the lot-specific COA.

	Sample ID	Calc. Conc. (pg/mL)	Inter-plate Calc. Conc. %CV	Avg. Intra-plate Calc. Conc. %CV	% Total Error
Kit Lot 1 N=2	Control 1	2586	9.7	5.1	13
	Control 2	683	10.1	7.0	19
	Control 3	210	8.5	8.0	9
Kit Lot 2 N=4	Control 1	2719	9.7	8.5	18
	Control 2	675	9.6	7.8	18
	Control 3	228	9.4	5.4	19
Kit Lot 3 N=23	Control 1	2453	12.4	6.8	14
	Control 2	611	16.6	10.0	19
	Control 3	205	10.9	6.6	13
Inter-Lot Summary N=29	Control 1	2499	12.1	7.0	
	Control 2	625	15.8	9.5	
	Control 3	208	11.1	6.5	

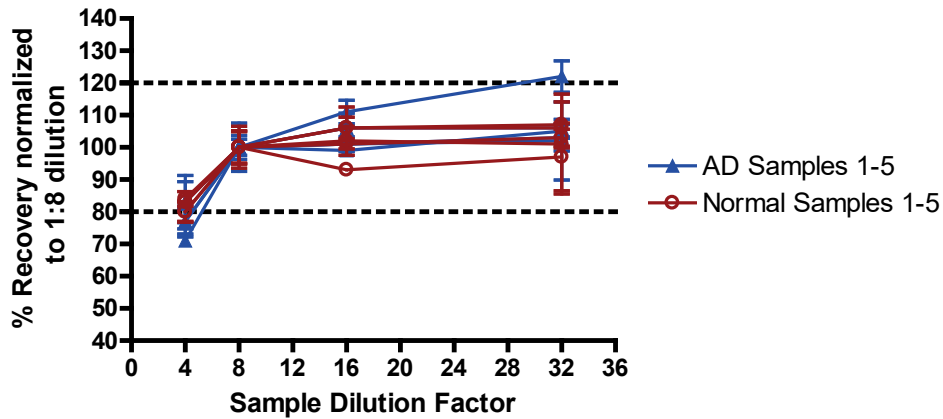
	% Conc. Recovery Relative to Final Expected Concentration		
	Kit Lot 1	Kit Lot 2	Kit Lot 3
Control 1	103	109	98
Control 2	109	108	98
Control 3	101	110	98

Dilution Linearity

To assess linearity, CSF from normal and Alzheimer’s disease (AD) individuals were diluted 4-fold, 8-fold, 16-fold, and 32-fold with Diluent 35. The measured concentrations were corrected for dilution factor to determine the actual Aβ42 levels in the sample. Recovery at each dilution was calculated relative to the optimal sample dilution (8-fold).

The average percent recovery and range of recovery for normal and AD samples at each dilution are presented in the graph and table below. A minimum sample dilution of 8-fold is recommended.

$$\% \text{ recovery} = (\text{measured} \times \text{dilution factor}) / (\text{measured at 8-fold dilution} \times 8) \times 100$$



Sample	Fold Dilution	Aβ42	
		Average %Recovery	%Recovery Range
Normal CSF (N=5)	4	82	80–84
	8	100	N/A
	16	102	93–106
	32	103	97–107
AD CSF (N=5)	4	78	71–83
	8	100	N/A
	16	104	99–111
	32	108	102–122

Spike Recovery

CSF from normal and individuals with Alzheimer's disease was spiked with calibrator at multiple levels throughout the range of the assay. The samples were then diluted 8-fold (as shown in the Sample section below) and tested for recovery.

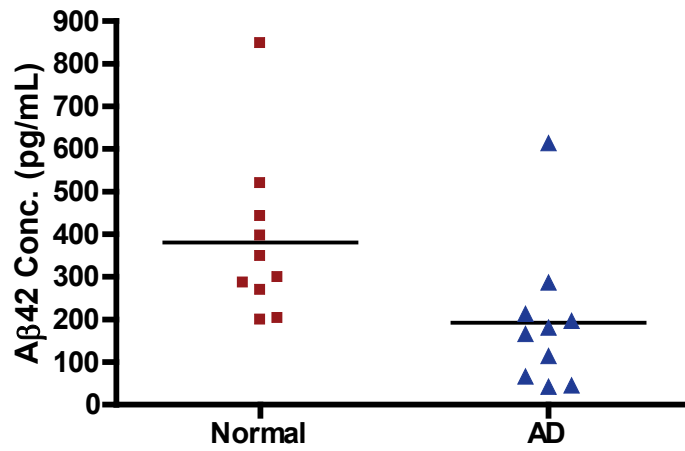
$$\% \text{ Recovery} = \frac{\text{measured concentration}}{\text{expected concentration}} * 100\%$$

Sample	Aβ42		
	Spike Conc. (pg/mL)	Average %Recovery	%Recovery Range
Normal CSF (N=5)	4000	88	81–92
	1000	98	92–105
	250	95	87–104
AD CSF (N=5)	4000	89	84–94
	1000	95	93–99
	250	95	90–103

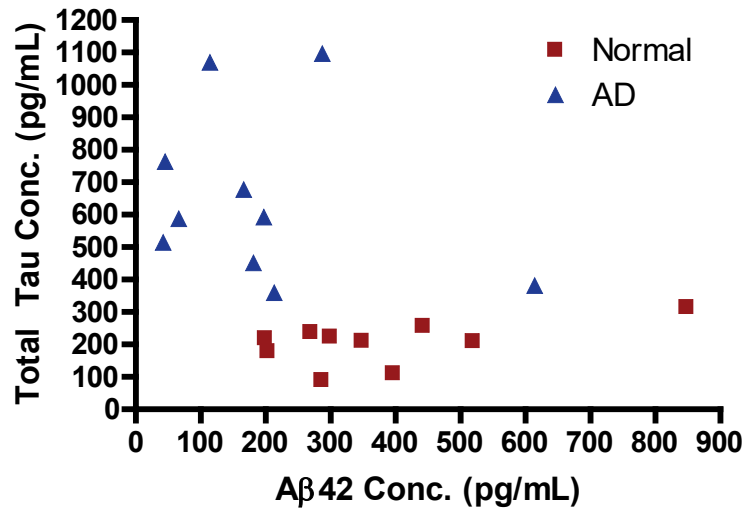
Tested Samples

CSF samples from normal and individual patients with AD as well as pooled human CSF samples were purchased from commercial vendors. Sample collection methods and pre-analytical variables may cause variability in the measured range of normal and diseased samples. The individual patient samples were well curated; handling was consistent with accepted protocols. The commercial vendors that supplied the pooled CSF samples were not able to adhere to stringent collection and handling procedures. Samples were diluted 8-fold before measuring with the Human Aβ42 Kit. The table below displays the median and range of concentrations for each sample set. Concentrations have been corrected for sample dilution. A graphical representation is also provided for the individual samples from normal individuals and individuals with AD.

			Aβ42 (pg/mL)
Well-curved, Individual, Human CSF Samples	Normal	Median (pg/mL)	324
		Range (pg/mL)	199–848
		No. of Samples	10
		% of Samples in Quantitative Range	100%
	AD	Median (pg/mL)	174
		Range (pg/mL)	42–614
		No. of Samples	10
		% of Samples in Quantitative Range	100%
Pooled Human CSF		Median (pg/mL)	92
		Range (pg/mL)	20–247
		No. of Samples	10
		% of Samples in Quantitative Range	80%



Evaluation of human Aβ42 levels in combination with other biomarkers can be a powerful tool for distinguishing sample populations. The plot below demonstrates the relationship between Aβ42 and total tau and levels in normal and AD samples.



Specificity

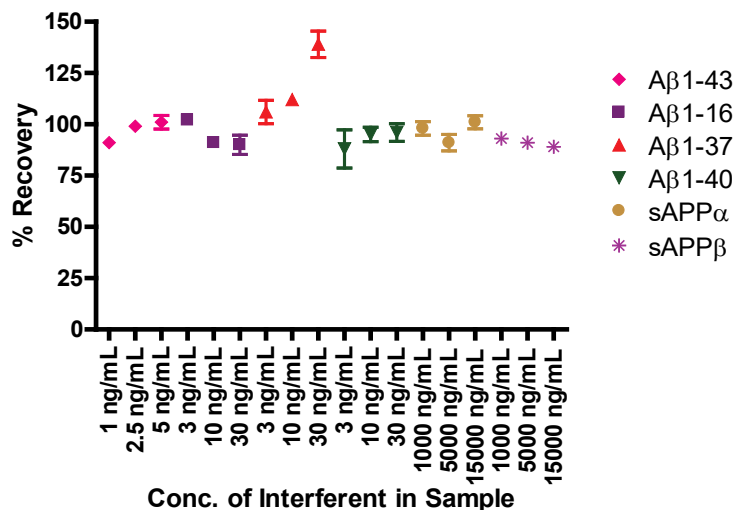
Different A β peptides and amyloid precursor proteins were spiked into Diluent 35 and tested with the Human A β 42 Kit. Concentrations shown in the table have been corrected for sample dilution.

The assay detects very low levels of A β 1-41, 1-43, and 1-37. Grey shading indicates values outside the limits of quantification of the A β 42 Assay. ND indicates not detected.

Analyte Spiked	Dilution Adjusted Conc. of Spiked Analyte (pg/mL)	% of Spiked Calibrator Recognized in the A β 42 Assay
A β 1-16	24 000	ND
A β 1-24	24 000	ND
A β 1-37	24 000	0.93
A β 1-38	24 000	ND
A β 1-39	24 000	0.03
A β 1-40	80 000	0.02
A β 1-41	24 000	0.22
A β 1-43	24 000	0.41
sAPP β	8 000 000	ND
sAPP β	8 000 000	ND

Interference

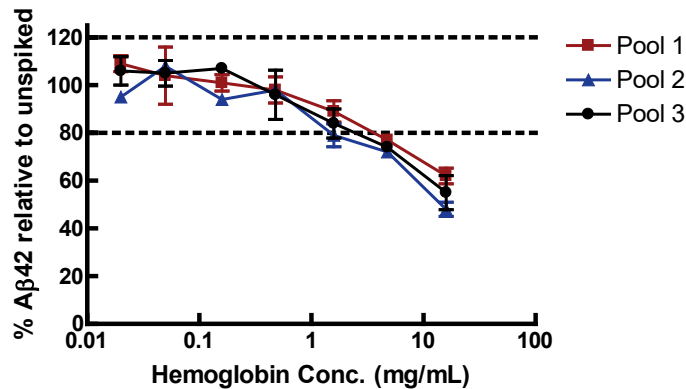
The Human A β 42 Kit was designed to minimize interferences in A β 42 measurement. Various A β peptides and amyloid precursor proteins were spiked into human CSF at levels that exceeded the expected endogenous levels for these analytes as noted below.¹⁰ The endogenous A β 42 levels were measured in the parent and spiked samples. Measured A β 42 levels were largely within 20% of the parent sample, regardless of the spiked analyte or concentration. Comparable results were observed when a low level of A β 42 calibrator was cospiked with excess A β peptides and amyloid precursor proteins in Diluent 35 (data not shown).



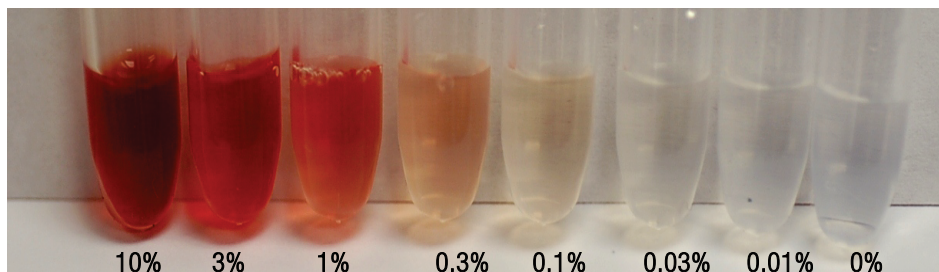
Effect of Hemolysis

The Human A β 42 Kit is tolerant of up to 1.6 mg/mL hemoglobin in CSF, which is equivalent to 1% blood contamination in the sample. Assay tolerance to blood contamination was assessed by measuring A β 42 levels in CSF spiked with hemolyzed clarified blood. The hemoglobin concentration in the hemolyzed blood sample was estimated through absorbance measurement at 414 nm (extinction coefficient 524,280 cm⁻¹/M). The measured concentration was 160 g/L hemoglobin, consistent with expected hemoglobin levels in normal whole blood.

Hemolyzed blood was titrated into three human CSF pools. The resulting contaminated samples contained 0.02–16 mg/mL hemoglobin, which is equivalent to 0.01–10% blood in the sample. Spiked samples were diluted 8-fold and tested with the Human A β 42 Kit. The measured A β 42 concentration relative to the unspiked sample is plotted below. Samples with 0.1% contamination were tinged slightly pink; samples with 1% contamination were dark pink and easily identified as contaminated.



% Blood Spike Level	Hemoglobin Conc.(mg/mL)	% Measured Concentration Relative to Unspiked Sample		
		Pool 1	Pool 2	Pool 3
10.0	16	62	48	55
3.00	4.8	77	72	74
1.00	1.6	89	79	84
0.300	0.48	98	98	96
0.100	0.16	101	94	107
0.030	0.048	104	108	105
0.010	0.016	109	95	106
0	0	100	100	100



Stability

The kit calibrator vials are stable up to 3 freeze/thaw cycles. The control samples (if purchased separately, catalog No. C41LB-1) are provided as single-use vials. Subjecting controls to multiple freeze-thaw cycles is not recommended.

Assay Components

Calibrator

Synthetic peptide built from human A β 42 sequence is used as the calibrator for the Human A β 42 Kit.

Antibodies

Analyte	Source Species		Assay Generation
	MSD Capture Antibody	MSD Detection Antibody	
A β 42	Mouse Monoclonal	Mouse Monoclonal	B

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6. Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res.* 2006;23:312-28.
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Summary Protocol

Human A β 42 Kit

*MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing
the Human A β 42 assay.*

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. Mix stock and diluted kit reagents thoroughly.

Sample and Reagent Preparation

- Prepare calibration 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 the 50X detection antibody to 1X in Diluent 35.
- Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Diluent 35

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

Step 2: Wash and Add Sample, Control, or Calibrator

- Wash plate 3 times with 300 μ L/well of PBS-T.
- Add 50 μ L/well of calibrator, diluted sample, or diluted control.
- Incubate at room temperature with shaking for 1 hour.

Step 3: Wash and Add Detection Antibody Solution

- Wash plate 3 times with 300 μ L/well of PBS-T.
- Add 25 μ L/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 300 μ L/well of PBS-T.
- Add 150 μ L/well of 2X Read Buffer T.
- Analyze plate on the MSD instrument.

Plate Diagram

