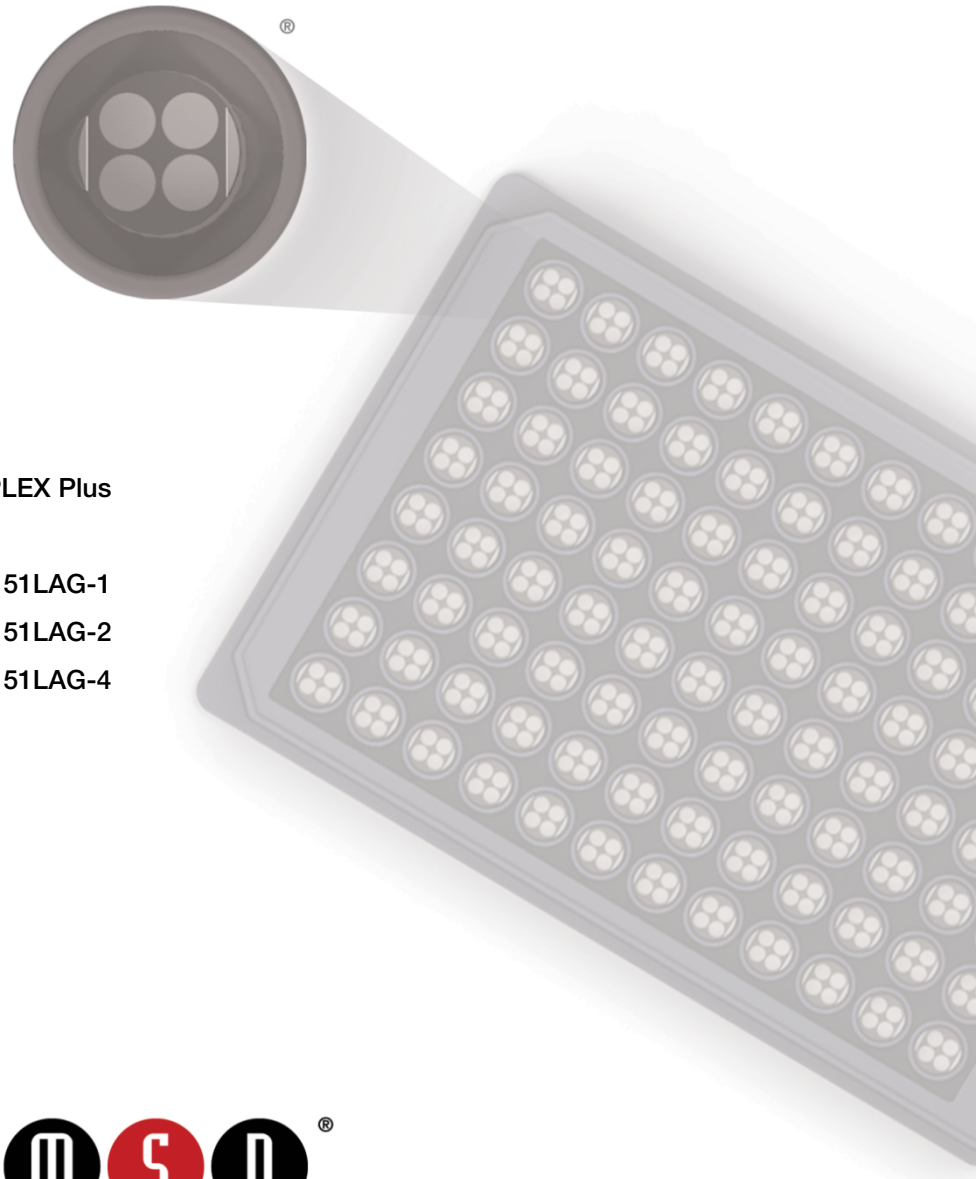
MSD® MULTI-SPOT Assay System

Human Total Tau Kit

V-PLEX®



	V-PLEX®	V-PLEX Plus
1-Plate Kit	K151LAE-1	K151LAG-1
5-Plate Kit	K151LAE-2	K151LAG-2
25-Plate Kit	K151LAE-4	K151LAG-4

MSD Neurodegenerative Disease Assays

Human Total Tau 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.

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Introduction

The human tau protein family consists of six isoforms which are alternately spliced from the MAPT gene. Tau is expressed primarily in neuronal cells where it localizes to the axon and impacts both cytoskeletal structure and cell signaling. Tau has emerged as a putative therapeutic target for many neurodegenerative disorders, including Alzheimer's disease (AD), frontotemporal dementia, Pick's disease, progressive supranuclear palsy, and corticobasal degeneration.[1] A key characteristic of these tauopathies is the presence of intracellular neurofibrillary tangles made up predominantly of hyperphosphorylated forms of the protein.

Tau and Aβ42 have been identified as core AD biomarkers. Their levels in cerebrospinal fluid (CSF) reproducibly distinguished samples from normal and AD individuals, and the combination may be useful in identifying samples from individuals with mild cognitive impairment (MCI) in AD research.[2-4] Studies aimed at evaluating the association between AD-type pathologic changes in the brain and antemortem CSF levels of Aβ42 and tau protein indicated that levels of both proteins correlated with the presence of neurofibrillary tangles and Aβ in the brain.[5] CSF total tau and Aβ42 levels are effective markers for discriminating incipient AD from age-related memory impairment, depression, and some secondary dementias; thus these biomarkers have proven useful in AD research.[6]

The MSD Human Total Tau Kit has been validated for the detection of total tau protein in CSF. The kit may also be used to measure total tau levels in cell lysates, conditioned cell culture media prepared from human neuronal cells grown in tissue culture, and human tissue homogenates. Standardized assays that have minimal variability across manufacturing runs, users, and platforms are needed for accurate analysis of AD markers.[7] MSD is committed to providing state-of-the-art biomarker measurements to the neurodegenerative disease research community and has independently 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 Total Tau 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 Total Tau is a sandwich immunoassay. MSD provides a plate that has been precoated with a capture antibody for total tau. The user adds the sample and a solution containing the detection antibody conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of 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.[8]

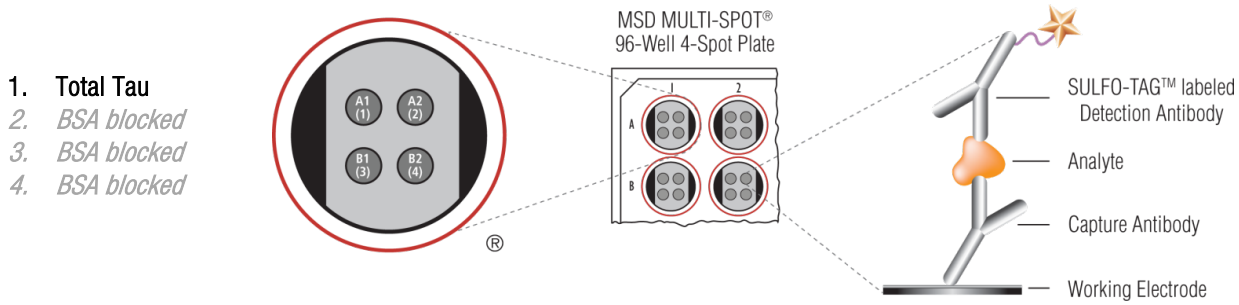


Figure 1. *Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.*

Kit Components

The Human Total Tau assay is available as 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 Total Tau Plate	2–8°C	N451LAA-1	4-Spot	1 plate	5 plates	25 plates	96-well plate, foil sealed, with desiccant.
Tau Calibrator (20X)	≤-70°C	C01LA-2	1 vial	1 vial	5 vials	25 vials	Recombinant tau protein in diluent that mimics human CSF. Analyte concentration is provided in the lot-specific certificate of analysis (COA).
SULFO-TAG Anti-Total Tau Antibody (50X)[1]	2–8°C	D21LA-2	75 µL	1 vial			SULFO-TAG conjugated antibody.
		D21LA-3	375 µL		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.

V-PLEX Plus Kits: Additional Components

Reagents	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Neurodegeneration Control 1*	2–8°C	C41LB-1	100 µL	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 COA.
Neurodegeneration Control 2*	2–8°C	C41LB-1	100 µL	1 vial	5 vials	25 vials	
Neurodegeneration Control 3*	2–8°C	C41LB-1	100 µL	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)

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

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 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 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.
- Bring frozen diluent(s) to room temperature in a 24°C water bath. Thaw other reagents on wet ice and use as directed without delay.
- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.
- 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 introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous with a rotary motion between 300 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 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 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 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 Total Tau Assay.

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

1) Vortex the thawed calibrator stock vial.
2) Prepare the highest calibrator by adding 20 µL of the 20X calibrator stock to 380 µL of Diluent 35. Mix well by vortexing. Repeat 3-fold serial dilutions 6 additional times to generate 7 calibrators.
3) Use Diluent 35 as the zero calibrator.

Alternative Calibration Curve: In certain sample populations, it may be advantageous to add an additional lower calibrator point on the curve.

For the lot-specific concentration of the calibrator, refer to the 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 total tau levels. MSD recommends reviewing current literature and protocols such as those proposed by the Alzheimer's Disease Neuroimaging Initiative (ADNI).[9] The samples described below were clarified with a single centrifugation step at 1,200 rcf for 10 minutes at 2–8˚C. Samples were then aliquotted and flash frozen to minimize potential freeze/thaw effects.

CSF samples measured using the Human Total Tau Kit exhibit good linearity; see the Dilution Linearity section for representative data. We recommend diluting human CSF samples 2- to 4-fold in Diluent 35; however, you may need to use higher or lower dilution factors depending on the sample set under investigation.

1) Vortex the thawed sample.
2) Dilute 2-fold or 4-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 recombinant total tau protein into a diluent that mimics human CSF.

Thaw controls at room temperature and mix well by vortexing. Dilute controls 4-fold in Diluent 35 and mix well by vortexing. For lot-specific concentrations of controls, 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 detection antibody solution is 1X.

For one plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-Total Tau 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 one 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 1X.

For one plate, combine:

- ❑ 5 mL Read Buffer T (4X)
- ❑ 15 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 first 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.

   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 incubate at room temperature with shaking for 1 hour.

   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 1X Read Buffer T to each well of the MSD plate. Incubate for 8–10 minutes without shaking at room temperature. Analyze the plate on the MSD instrument.

   Note: If using the SECTOR® Imager 6000, the MESO® SECTOR S 600, or the MESO QuickPlex® SQ 120, sensitivity may be improved by reading immediately after adding the read buffer.

Validation

The Human Total Tau Kit was validated for the detection of total tau protein 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.[8] 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 tau 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 in order to assess kit sensitivity, accuracy, and precision. Spike recovery and dilution linearity were assessed using samples from normal and AD individuals. Assay specificity and tolerance to sample contamination with hemolyzed blood was 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. We describe specifications for and representative data from these tests in the sections below.

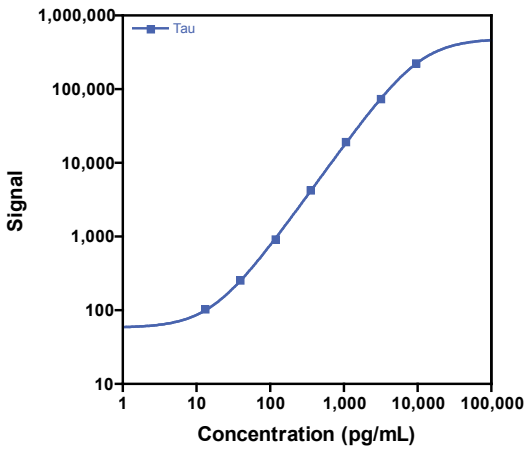
Analysis of Results

The calibration curve is modeled using least-squares fitting algorithms to calculate the concentration of 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.

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 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.



	Tau[2]	
Conc. (pg/mL)	Average Signal	%CV
0	57	17.1
13.2	103	6.8
39.5	253	6.0
119	913	6.5
356	4,230	7.3
1,067	19,164	6.2
3,200	73,507	7.3
9,600	223,102	6.2

[2] See the kit-specific COA for calibration curve concentrations, specification, 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 three days of testing (N=54 runs across three kit lots). A summary of the Human Total Tau Assay sensitivity and dynamic range is presented in the table below. In-well concentrations are reported.

The 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=54 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% to 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% to 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 for download at www.mesoscale.com.

	Tau (pg/mL)
LLOD Range	1.07– 23.7
LLOQ	30.0
ULOQ	8,000

Precision

Control samples using pooled human CSF with or without spiked tau calibrator were built. Two sets of control samples were independently prepared and tested in the Human Total Tau Kit. Each set contained three controls with total tau levels spanning the expected range of total tau in human CSF samples. Controls were diluted 4-fold. Concentrations for all controls were measured using three independent Human Total Tau Kit lots. Representative data from one set of controls is presented in the tables below. For this study, four analysts ran tests over ten days (N=26 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. Avg. Intra-plate Calc. Conc. %CV is the average concentration CV of the control replicates on an individual plate. Inter-plate 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 (Inter-plate 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 concentrations for each control. Measured concentrations for each kit relative to the final expected concentrations are presented in the lower table.

The controls had low variability (CVs <20%), and the control concentrations measured on 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=3	Control 1	4,928	5.0	3.3	12
	Control 2	1,167	3.0	2.5	3
	Control 3	304	6.3	3.2	9
Kit Lot 2 N=5	Control 1	4,734	7.2	3.1	10
	Control 2	1,118	2.8	3.3	7
	Control 3	260	4.8	5.4	17
Kit Lot 3 N=18	Control 1	4,504	9.5	2.6	12
	Control 2	1,184	7.2	5.3	8
	Control 3	305	18.6	5.0	21
Inter-Lot Summary N=26	*Control 1*	*4,598*	*9.0*	*2.4*	
	Control 2	*1,170*	*6.5*	*4.3*	
	Control 3	*296*	*17.1*	*4.5*	

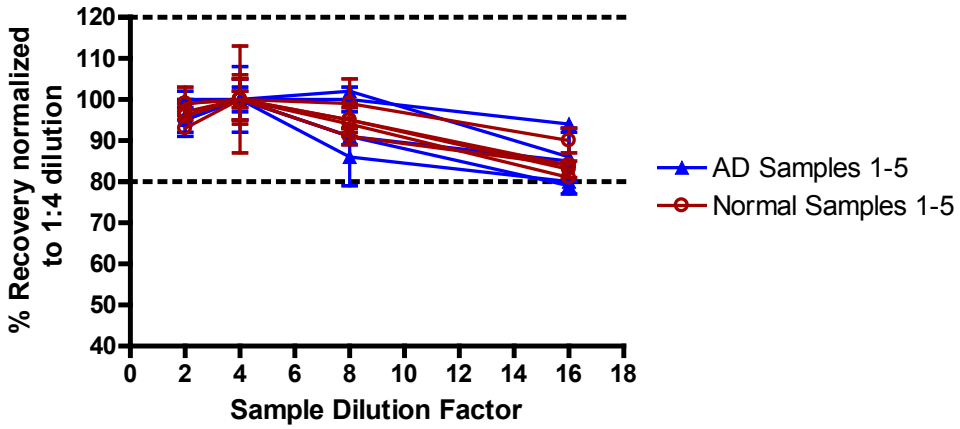
	% Conc. Recovery Relative to Final Expected Concentration		
	Kit Lot 1	Kit Lot 2	Kit Lot 3
Control 1	107	103	98
Control 2	100	96	101
Control 3	103	88	103

Dilution Linearity

To assess linearity, CSF samples from normal and AD individuals were diluted 2-fold, 4-fold, 8-fold, and 16-fold with Diluent 35. Measured concentrations were corrected for dilution factor to determine the actual total tau levels in the sample. Recovery at each dilution was calculated relative to the optimal sample dilution (4-fold).

Average percent recovery and range of recovery for normal and AD samples at each dilution are presented in the graph and table below. The graph of percent recovery versus dilution factor shows that a 2-fold dilution may be used for higher sensitivity with minimal effect on recovery. A minimum sample dilution of 2-fold is recommended.

% Recovery=(measured∗dilution factor)/(measured at 4-fold dilution∗4)∗100



		Tau	
Sample	Fold Dilution	Average %Recovery	%Recovery Range
Normal CSF (N=5)	2	97	92–99
	4	100	N/A
	8	94	87–96
	16	89	83–93
AD CSF (N=5)	2	96	92–98
	4	100	N/A
	8	94	89–99
	16	87	83–93

Spike Recovery

CSF from normal and AD individuals was spiked with calibrator at multiple levels throughout the range of the assay. The samples were then diluted 4-fold (as shown in the Sample section below) and tested for recovery.

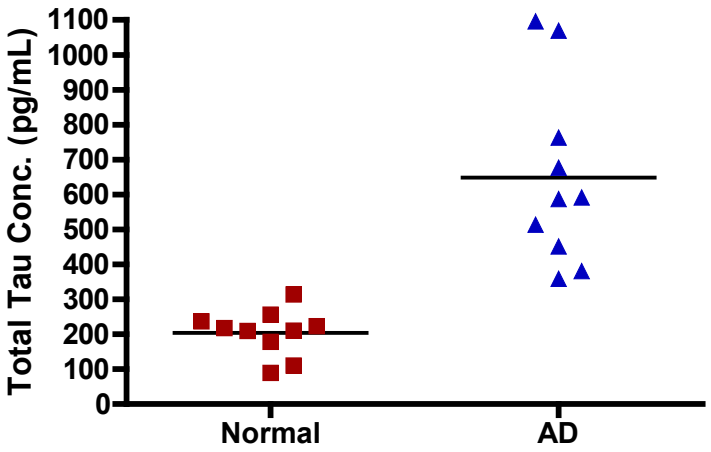
$$\%\ Recovery = \frac{measured\ concentration}{expected\ concentration} * 100\%$$

	Tau		
Sample	Spike Conc. (pg/mL)	Average %Recovery	%Recovery Range
Normal CSF (N=5)	4,000	101	93–107
	1,000	110	106–113
	250	105	99–109
AD CSF (N=5)	4,000	101	97–104
	1,000	112	106–116
	250	106	104–107

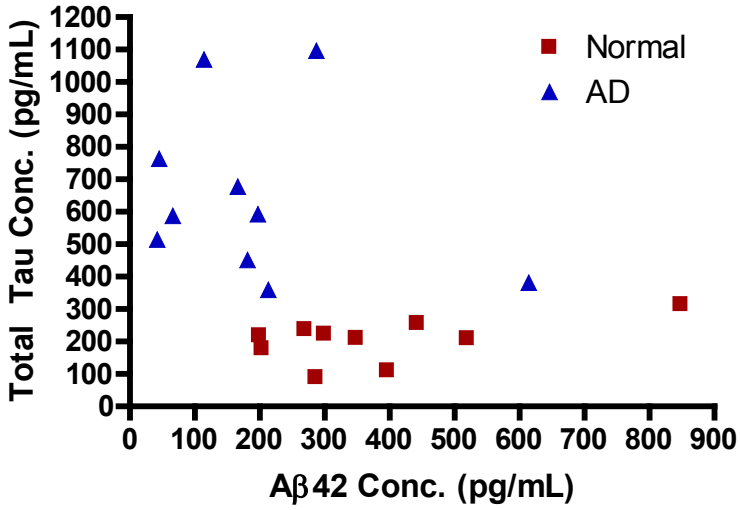
Tested Samples

Individual CSF samples from normal and AD individuals and 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 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 4-fold prior to measuring with the Human Total Tau Kit. Aβ42 concentration in the samples was measured using MSD Human Aβ42 Kit. The table below displays 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 normal and AD samples.

			Tau (pg/mL)
Well-curated, Individual, Human CSF Samples	Normal	Median (pg/mL)	214
		Range (pg/mL)	89–314
		# of Samples	10
		% of Samples in Quantitative Range	80%
	AD	Median (pg/mL)	590
		Range (pg/mL)	359–1,096
		# of Samples	10
		% of Samples in Quantitative Range	100%
Pooled Human CSF		Median (pg/mL)	1,560
		Range (pg/mL)	105–10,756
		# of Samples	10
		% of Samples in Quantitative Range	90%



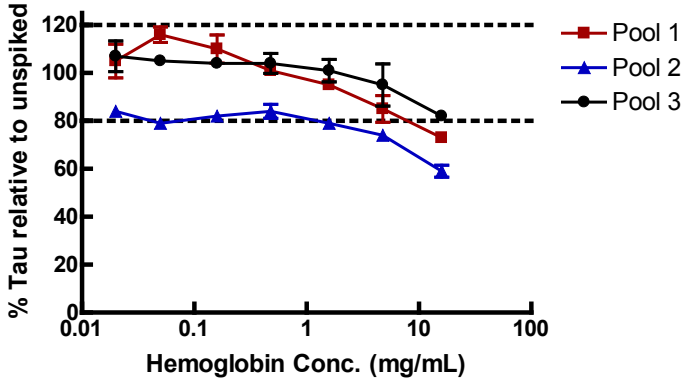
Evaluation of human total tau levels in combination with other biomarkers can be a powerful tool for distinguishing sample populations. The plot below demonstrates the relationship between total tau and Aβ42 levels in normal and AD samples.



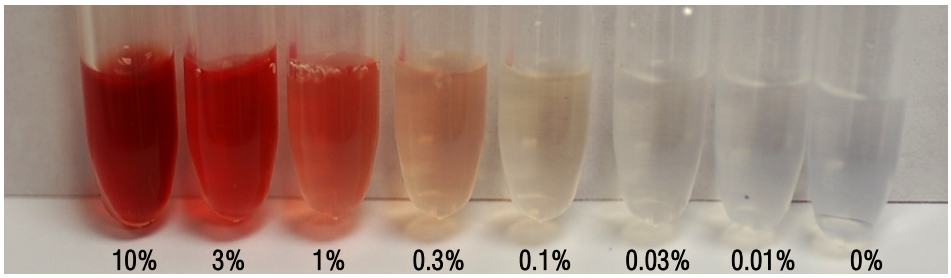
Effect of Hemolysis

The Human Total Tau 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 total tau 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 4-fold and tested with the Human Total Tau Kit. The measured total tau concentration relative to the unspiked sample is plotted below. Samples with 0.1% contamination are tinged slightly pink; samples with 1% contamination are dark pink and easily identified as contaminated.



		% Measured Concentration Relative to Unspiked Sample		
% Blood Spike Level	Hemoglobin Conc.(mg/mL)	Pool 1	Pool 2	Pool 3
10.0	16	73	59	82
3.00	4.8	85	74	95
1.00	1.6	95	79	101
0.300	0.48	101	84	104
0.100	0.16	110	82	104
0.030	0.048	116	79	105
0.010	0.016	105	84	107
0	0	100	100	100



Specificity

Given the reported binding sites for the capture and detection antibodies used in the Human Total Tau Kit, binding to all six isoforms of human tau is expected (refer to the table in the Assay Components section). As part of the validation of the Human Total Tau Kit, binding of the six tau isoforms was confirmed on each of the three independently built kit lots.

The Human Total Tau Kit did not show any cross-reactivity with mouse samples when tested with brain homogenates.

Stability

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

Assay Components

Calibrator

Full length recombinant tau (isoform tau441) protein expressed in *E. coli* is used as calibrator for the Human Total Tau Kit.

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
Tau	Mouse Monoclonal	Mouse Monoclonal	B

Isoforms of Tau Recognized by Antibodies

Isoforms Recognized	
MSD Capture Antibody	MSD Detection Antibody
Tau352, 381, 383, 410, 412, 441	Tau352, 381, 383, 410, 412, 441

References

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4. Blennow K, et al. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol. 2010;6:131-44.
5. Tapiola T, et al. Cerebrospinal Fluid β-Amyloid 42 and Tau Proteins as Biomarkers of Alzheimer-Type Pathologic Changes in the Brain. Arch Neurol. 2009;66:382-9.
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8. Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006;23:312-28.
9. Alzheimer's Disease Neuroimaging Initiative Procedures Manuals. Website: http://www.adni-info.org/Scientists/ProceduresManuals.aspx.

Summary Protocol

Human Total Tau Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Total Tau 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 50-fold in Diluent 35.
- ❑ Prepare 1X Read Buffer T by diluting 4X Read Buffer T 4-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 Sample, Control, or Calibrator

- ❑ Wash plate 3 times with at least 150 µL/well of PBS-T.
- ❑ Add 50 µL/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 at least 150 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with shaking for 1 hour.

Step 4: Wash and Read Plate

- ❑ Wash plate 3 times with at least 150 µL/well of PBS-T.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Incubate at room temperature without shaking for 8–10 minutes. Analyze plate on the MSD instrument.

Note: If using SECTOR Imager 6000, the MESO SECTOR S 600, or the MESO QuickPlex SQ 120, sensitivity may be improved by reading immediately after adding the read buffer.

Plate Diagram