MSD® MULTI-SPOT Assay System

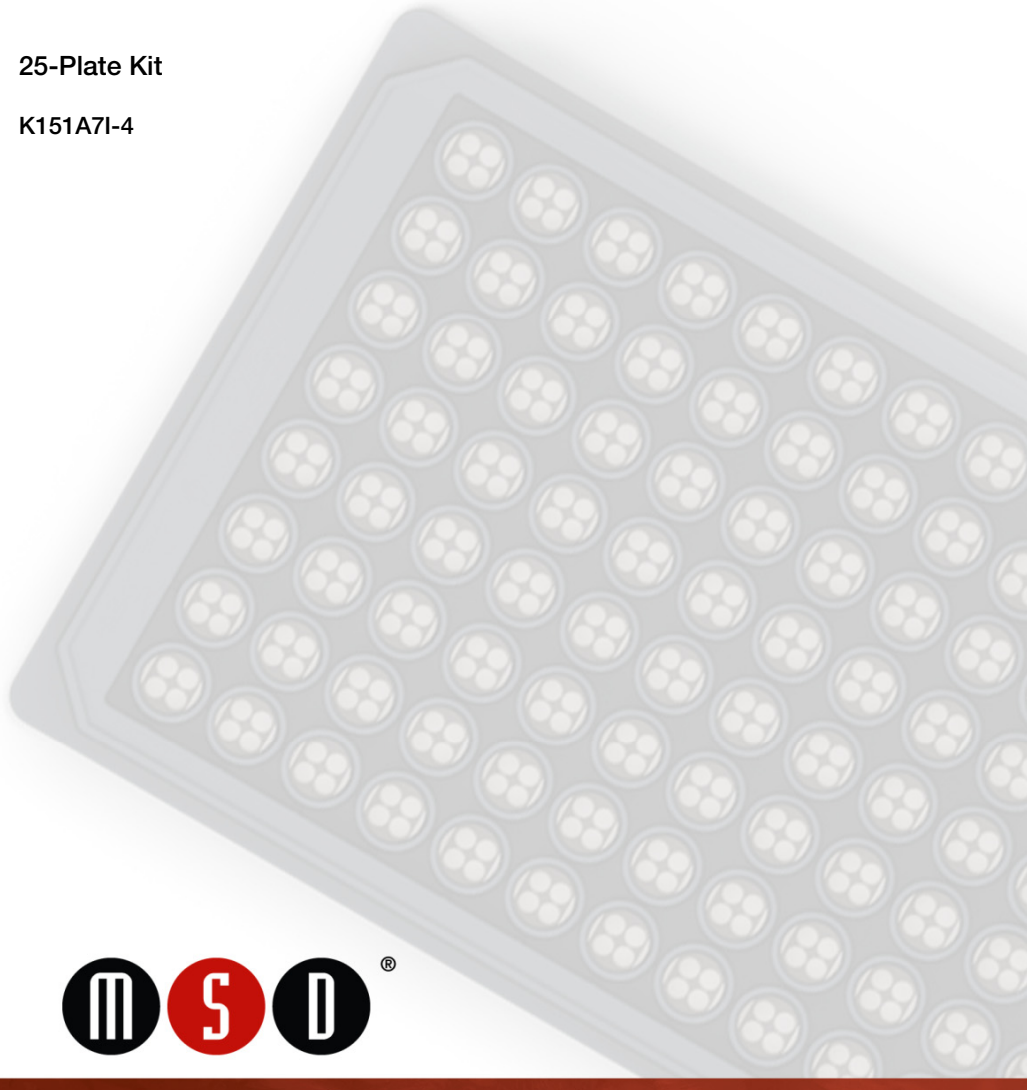
Special Order Human Neurodegeneration Group 1

V-PLEX™ Assays: Aβ38, Aβ40, Aβ42, Tau

Standard Assays: α-Synuclein, DJ-1/PARK7, sAPPα, sAPPβ, Swedish sAPPβ

For assay conversion: Streptavidin-Coated Spot

Special Order Kits	5-Plate Kit	25-Plate Kit
Catalog #	K151A7I-2	K151A7I-4



MSD Neurodegeneration Assays

Special Order Human Neurodegeneration Group 1 Kits

Aβ38, Aβ40, Aβ42, Tau, α-Synuclein, DJ-1/PARK7, sAPPα, sAPPβ, Streptavidin-Coated Spot

For use with cerebrospinal fluid (CSF)

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

Special order kits allow customers to combine validated V-PLEX assays in new configurations or to mix V-PLEX assays with selected assays from MSD's standard menu. This allows customers to create unique multiplex assays for their specific applications. Special order reagents for V-PLEX assays are the same as those provided in the pre-configured V-PLEX kits, including calibrators. Calibrators for standard assays are supplied from the same bulk materials that are used for standard MSD assays.

The assays listed below have been tested by MSD for analytical compatibility; data on analytical performance, calibration curves, reproducibility, and specificity are provided in the certificate of analysis (C of A) included with each kit. Special order applications may be unique; therefore, users should test abundance levels in their specific samples to determine the optimum combination of assays.

MSD assays that can be combined into a Special Order Neurodegeneration Group 1 are:

Human V-PLEX Assays: Aβ38, Aβ40, Aβ42, Human Total Tau

Analytically Compatible, Standard Human Assays: α-Synuclein, DJ-1/PARK7, sAPPα, sAPPβ, Swedish sAPPα.

A streptavidin-coated spot can be included in this special order group to facilitate the transfer of customer-developed ELISAs to the MSD platform. See Alternate Protocols and the Appendix for more information on converting an ELISA.

Principle of the Assay

MSD neurodegeneration assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. These assays are all sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots, as shown in the layout below. The assays are provided on MSD 4-spot, 7-spot, or 10-spot plates. 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 to provide a quantitative measure of analytes in the sample.

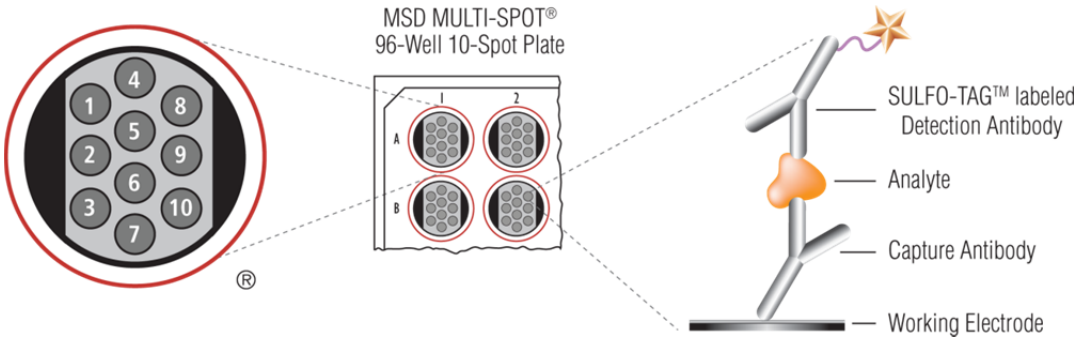

Figure 1: Multiplex plate spot diagram showing possible placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Figure 2: Special order assays can also be provided on 4- or 7-spot plates.

A spot map identifying the location of each assay ordered can be found on the plate packaging. This information will be needed for data analysis.

Reagents Supplied

Reagent	Storage	Catalog #	Description
MULTI-SPOT® 96-Well Plate	2–8°C	—	4-, 7-, or 10-spot, 96-well plate (depending on assays ordered), pre-coated with specific capture antibodies for each assay ordered; foil sealed, with desiccant.
V-PLEX assay calibrator	≤-70°C	C00NZ-2 - Aβ 1-38 Peptide C00OA-2 - Aβ 1-40 Peptide C01LB-2 - Aβ 1-42 Peptide C01LA-2 - Tau	Individual calibrator for each V-PLEX assay ordered; sufficient for 5 plates. The Aβ peptide and tau calibrators contain synthetic Aβ peptides or tau protein in diluent that mimics human CSF. Analyte concentrations are provided in the lot-specific C of A.
Standard assay calibrator (50 µg/mL)	≤-70°C	—	Individual calibrator for each standard assay ordered; one vial per assay; sufficient for 5 plates. Analyte concentrations are provided in the lot-specific C of A.
Diluent 35*	2–8°C	R50AE-3 R50AE-2	Diluent for samples and calibrator; mimics human CSF; contains proteins and preservatives; sufficient for 5 plates.*
Diluent 100	2–8°C	R50AA-4	Diluent for detection antibody; contains protein, blockers, and preservatives; sufficient for 5 plates.
Read Buffer T (4X)	RT	R92TC-3	Buffer to catalyze the electrochemiluminescence reaction.
Individual detection antibody for each assay ordered (50X)**	2–8°C	—	Individual detection antibody, SULFO-TAG–conjugated; 1 vial for each assay ordered; sufficient for 5 plates.
Aβ40 Blocker (10X)	2–8°C	R93BJ-2	Only supplied with kits that include the Aβ40 assay. Blocking reagent for measuring CSF samples containing high abundance of Aβ40 peptide, not for use with serum and plasma.

*Additional diluent may be purchased from MSD for sample dilution

**SULFO-TAG Anti-Aβ 6E10 antibody is provided as the detector for all 3 Aβ peptide assays. For multiplexes that combine sAPPα with Aβ peptide assays, SULFO-TAG Anti-Aβ 6E10 antibody is provided as the detector for sAAPα as well.

Reagents for Transferring an Assay to the MSD platform

A streptavidin-coated spot can be included in the special order to facilitate the transfer of customer-developed ELISAs to the MSD platform.

Capture reagents. Biotinylated capture reagents are required to transfer a self-developed ELISA to the MSD platform using the streptavidin-coated spot. Use ≤2 µg/mL of biotinylated reagent for each 25 µL of capture antibody solution. Suggested working concentrations range from 0.2 to 2 µg/mL for initial assay development. Many customers use 25 µL of a 1 µg/mL biotinylated capture reagent or 50 µL of a 0.5 µg/mL biotinylated capture reagent.

Detection antibody. When transferring a self-developed ELISA to the MSD platform, the suggested working concentration for the detection antibody ranges from 0.2 to 2 µg/mL for initial development. As a starting point, MSD recommends diluting the SULFO-TAG-conjugated detection antibody to 1 µg/mL and incubating for 1 hour following sample incubation.

See Alternate Protocols and the Appendix for more information on converting an ELISA.

Limitations of Use

- Either the sAPPα or the sAPPβ assay (but not both together) can be multiplexed with the Aβ peptide assays.
- Some assays may require greater dilution. (See Dilute Samples in the Reagent Preparation section below.)

Required Materials and Equipment (Not Supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1000 rpm
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog # R61AA-1
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex

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 safety data sheet (SDS), which can be obtained from MSD Customer Service.

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots. Lot information is provided in the lot-specific C of A.
- Bring frozen diluent(s), if any, to room temperature in a 24°C water bath. Thaw other reagents on wet ice and use as directed without delay.
- 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 introduction of bubbles, and for empty wells, pipette to the bottom corner.
- When adding reagents to the plate, ensure that the liquid completely covers the bottom of the well.
- 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 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 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. Vortex calibrators.

Prepare Calibrator Dilutions

MSD supplies individual frozen calibrators for each assay included in the special order. The following table lists the concentration of the provided stock calibrator for each analyte relative to the recommended highest calibrator concentration. The actual concentration of each calibrator stock and the recommended highest calibrator concentration are provided in the C of A included with the kit. Each vial of calibrator is sufficient for 5 plates.

Assay	Stock concentration provided relative to Calibrator 1	Calibrator 1 concentration (pg/mL)
Aβ1-38 Peptide	40X	Refer to C of A
Aβ1-40 Peptide	40X	Refer to C of A
Aβ1-42 Peptide	40X	Refer to C of A
Tau	20X	Refer to C of A
α-Synuclein	5000X	10 000
DJ-1/PARK7	500X	100 000
sAPPα	50X	1000 000
sAPPβ	50X	1000 000
Swedish sAPPβ	50X	1000 000

Prepare calibrator 1 by combining the individual calibrators supplied as described below, then prepare serial dilutions to create 7 calibrators plus a zero calibrator. If your special order does not include α-synuclein and/or DJ-1, proceed directly to Step 2.

Step 1 (for orders that include α-synuclein and DJ-1 only). The stock calibrators for α-synuclein and DJ-1 require an intermediate dilution prior to the preparation of Calibrator 1. Prepare a working stock (50X) of α-synuclein and DJ-1 calibrator as shown in Table A below.

Assay	Bulk Calibrator (µL)	Diluent 35 (µL)	50X Working Stock Concentration (µg/mL)
α-Synuclein	10	990	0.5
DJ-1/PARK7	20	180	5

Table A: Preparation of 50X Working Stock

Step 2. Prepare 7 calibrator solutions plus a zero calibrator blank:

1) Prepare Calibrator 1 by combining the supplied calibrators in the amounts listed in Table B and bringing the final volume to 500 µL with Diluent 35.

Assay	V-PLEX assay calibrators (µL)	50X Working Stock (µL)	Provided Stock Calibrator (µL)	Diluent 35 (µL)
Aβ1-38 Peptide	12.5			Add Diluent 35 to a final volume of 500 µL
Aβ1-40 Peptide	12.5			
Aβ1-42 Peptide	12.5			
Tau	25			
α-Synuclein		10		
DJ-1/PARK7		10		
sAPPα			10	
sAPPβ			10	
Swedish sAPPβ			10	

Table B: Preparation of Calibrator 1

2) Prepare Calibrator 2 by transferring 100 µL of Calibrator 1 to 300 µL of Diluent 35. Mix well. Repeat 4-fold serial dilutions 5 times to generate 7 calibrators.

3) Use Diluent 35 as Calibrator 8.

4) Discard any unused, diluted calibrator.

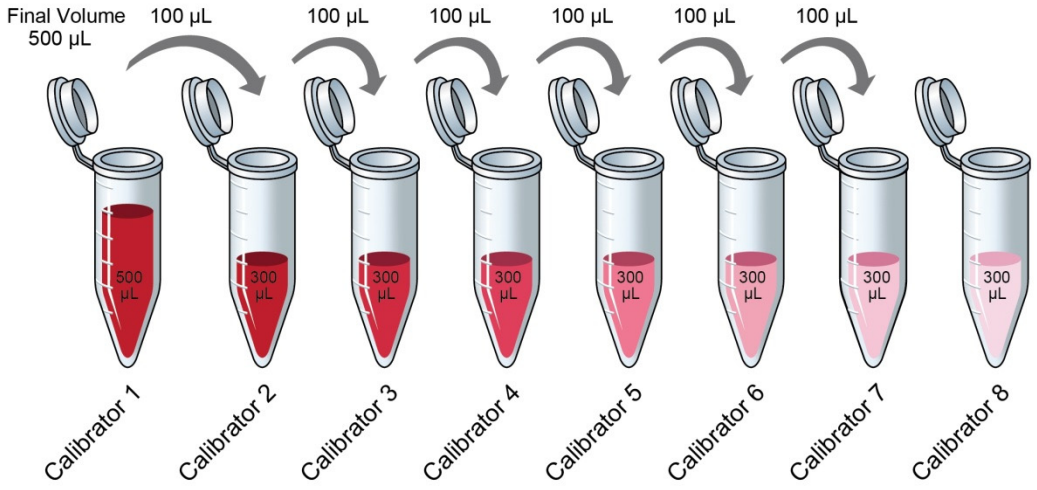

Figure 3: Typical dilution cascade. Adjust Calibrator 1 for assays ordered.

Dilute Samples

Dilute samples with Diluent 35. The recommended dilution factors for CSF samples are provided in the table below. The suggested dilution factors are based on the abundance of analyte in the sample, not on matrix effects. We recommend the dilution factors below; however, you may adjust dilution factors for the sample set under investigation. For example, to dilute 2-fold, add 60 µL of sample to 60 µL of Diluent 35. You may conserve sample volume by using a higher dilution.

		Suggested Fold Dilution
	Assay	CSF
V-PLEX Assays	Aβ38	2
	Aβ40	2
	Aβ42	2
	Tau	4
Standard Assays	α-Synuclein	8
	DJ-1/PARK7	8
	sAPPα	8
	sAPPβ	8
	Swedish sAPPβ	8

Prepare Aβ40 Blocker Solution

For kits that include the Aβ40 assay, the Aβ40 blocker may be included in the sample incubation step. For sample types that are expected to have low levels of Aβ40 peptide, Aβ40 blocker may be omitted.

MSD provides Aβ40 Blocker in a 10X stock solution. The working solution is 1X. Prepare the Aβ40 Blocker solution immediately prior to use.

For 1 plate, combine 300 µL of Aβ40 Blocker (10X) with 2700 mL of Diluent 35.

Prepare Detection Antibody Solution

MSD provides individual detection antibody for each assay ordered in a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use.

For 1 plate, combine 60 µL of each supplied 50X detection antibody, then add Diluent 100 to bring the final volume to 3000 µL.

Prepare Read Buffer

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

For 1 plate, combine:

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

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

Prepare MSD Plate

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

Protocol

If transferring a self-developed ELISA to the MSD platform using the streptavidin-coated spot, please see the Alternate Protocols section below.

1. **Block Plate:** 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.

2. **Wash and Add Sample:** Wash the plate 3 times with at least 150 µL/well of wash buffer.

   If Aβ40 Blocker is included, add 25 µL of diluted Aβ40 Blocker solution to each well. Then add 25 µL of diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

   If Aβ40 Blocker is not included, add 25 µL of Diluent 35 to each well. Then add 25 µL of diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

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

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

Alternate Protocols

Alternate Protocol A. For converting a self-developed ELISA using the streptavidin spot

Note: To incorporate a self-developed assay using the streptavidin-coated spot, you must first conjugate the detection antibody with SULFO-TAG, a necessary component of all MSD assays. Either the primary detection reagent can be directly conjugated with MSD SULFO-TAG NHS-ester, or a pre-labeled secondary reporter such as a SULFO-TAG anti-species antibody can be coupled to an unlabeled detection reagent. The protocol for SULFO-TAG conjugation can be found at www.mesoscale.com.

Following is an example of a typical assay protocol when using a streptavidin-coated spot for assay conversion:

1. Prepare biotinylated capture antibody using Diluent 100. Prepare 25 µL of antibody solution per well. For calculation of capture antibody concentration, please refer to the Appendix.

   Note: Solutions containing biotin should be avoided as free biotin can interfere with the binding of biotinylated capture reagents to streptavidin- and avidin-coated plates. Examples of blocking solutions containing biotin are milk-based blockers and certain cell culture media such as RPMI 1640.

2. Wash plate three times with PBS + 0.05% Tween (PBS-T). Dispense 25 µL/well of biotinylated capture antibody into the bottom of each well. Seal the plate and incubate at room temperature with shaking until binding equilibrium is achieved (usually 1 hour because binding interaction between biotin and streptavidin/avidin is very rapid). Prepare calibrators and controls during this time.

3. Wash plate three times with PBS + 0.05% Tween (PBS-T). Add sample as per the standard protocol above.

4. Wash plate three times with PBS + 0.05% Tween (PBS-T). Add detection antibody solution as per the standard protocol above.

5. Wash. Read plate as specified in the standard protocol.#

The suggestions below may be useful for simplifying the protocol; however, they have not been tested by MSD.

Alternate Protocol B: Extended Sample Incubation

Incubating samples overnight at 2–8°C may improve sensitivity for some assays. Shaking may improve performance.

Alternate Protocol C: Homogenous Incubation of Samples and Detector

A homogenous assay format may be tested. After the blocking step, wash the plate. Add 25 µL of detection antibody solution to each well. Then add 25 µL of diluted sample, calibrator, or control per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours. In kits that include the 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.

After incubation, wash the plate, add read buffer, and read plate.

Assay Components

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
Aβ38	Mouse Monoclonal	Mouse Monoclonal	A
Aβ40	Mouse Monoclonal	Mouse Monoclonal	A
Aβ42	Mouse Monoclonal	Mouse Monoclonal	B
Tau	Mouse Monoclonal	Mouse Monoclonal	C
α-Synuclein	Rabbit Monoclonal	Mouse Monoclonal	A
DJ-1/PARK7	Rat Monoclonal	Goat Polyclonal	A
sAPPα	Mouse Monoclonal	Mouse Monoclonal	A
sAPPβ	Rabbit Polyclonal	Mouse Monoclonal	A
Swedish sAPPβ	Rabbit Polyclonal	Mouse Monoclonal	A

C of A for Special Order Neurodegeneration Assays (human)

C of As for special order assays include the following:

- Signal and %CV from calibration curve for each calibrator
- Background signal
- Non-specific binding for all analytes (individual detection antibodies tested with blended calibrator)
- List of components with lot numbers used for QC testing

Appendix

Calculations for Capture Antibody Concentration

1. Converting picomoles of biotinylated protein/well to µg/mL concentration

$$\mu g/mL\ protein = \frac{pmol\ of\ biotinylated\ protein\ per\ well * molecular\ weight\ of\ protein\ (Da)}{(\mu L\ biotinylated\ protein\ added\ to\ well * 1000)}$$

The following example illustrates calculating the concentration of a 150 kD biotinylated antibody in µg/mL, such that 0.25 picomole is added to each well in a volume of 25 µL.

- picomole per well=0.25
- Antibody molecular weight (Da)=150 000
- Volume of biotinylated antibody added to well (µL)=25

µg/mL protein=0.25∗150000/(25∗1000)=1.5 µg/mL

2. Converting µg/mL biotinylated protein to picomole concentration

$$pmol\ protein = \frac{\mu g/mL\ concentration\ of\ protein * volume\ (\mu L) * 1000}{protein\ molecular\ weight\ (Da)}$$

- Antibody molecular weight (Da)=150 000
- Volume (µL)=25
- Concentration of protein (µg/mL)=1

pmol protein=1∗25∗1000/150000=0.167 pmol

3. Converting nM biotinylated protein to µg/mL concentration

$$\mu g/mL\ protein = \frac{nM\ concentration\ of\ protein * molecular\ weight\ of\ protein\ (Da)}{1\ 000\ 000}$$

- Antibody molecular weight (Da)=150 000
- Concentration of protein (nM)=15

µg/mL protein=15∗150000/1000000=2.2 µg/mL