

# MSD<sup>®</sup> $\beta$ -amyloid Peptide Assay: A $\beta$ 1-40

MULTI-SPOT<sup>®</sup> 4, 96-well Custom Plate

Storage

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## MSD Catalog Items

- ❑ MSD Read Buffer T (4X) RT

## R&D Custom Materials

- ❑ MULTI-SPOT 4, 96-well Custom plates 4°C
- ❑ MSD Tris Wash Buffer (10X) 4°C
- ❑ MSD Tris Lysis Buffer (1X) 4°C
- ❑ 3 pH test strips RT
- ❑ Silanized tubes 1.7 mL (for peptide storage) RT
- ❑ MSD SULFO-TAG<sup>™</sup> streptavidin 4°C
- ❑ Biotinylated detection antibody 4°C
- ❑ MSD Blocker D-R (10%) 4°C
- ❑ MSD Blocker A (dry powder) 4°C
- ❑ 10X peptide solvent -20°C
- ❑ A $\beta$ 1-40 peptide (dry) -20°C

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## Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Tris Wash Buffer and Read Buffer
- ❑ One 250 mL bottle
- ❑ Two 50 mL tubes
- ❑ Five 15 mL tubes
- ❑ Microcentrifuge tubes for making dilutions of peptide standards (silanized tubes are strongly recommended)
- ❑ Automated plate washer, Multidrop<sup>®</sup>, or other efficient multi-channel pipetting equipment for washing 96 well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25  $\mu$ L and 150  $\mu$ L into a 96-well micro plate

## Protocol at a Glance

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify A $\beta$ 1-40 peptide. The protocol takes approximately 4 hours to complete if each reagent is prepared during the preceding incubation. All reagents, with the exception of the diluted peptide, can be prepared ahead of time. This lengthens the overall time required but frees up time during the incubation steps.

Once desired results are achieved, the protocol can be streamlined to eliminate multiple incubation and wash steps to increase throughput.

1. Add MSD Blocker A Solution, incubate 1 hour, wash.
2. Add peptide, incubate 1 hour, wash.
3. Add detection antibody, incubate 1 hour.
4. Add SULFO-TAG streptavidin, incubate 1 hour, wash.
5. Add Read Buffer and analyze plate.

## Detailed Instructions

*Prepare a stock of 1X Tris Wash Buffer:*

- a) 1X Tris Wash Buffer is used throughout the assay to make other reagents as well as to wash plates. Approximately 250 mL per plate is required – more if using an automatic plate washer to account for waste.
- b) In a 250 mL bottle combine:
  - 25 mL 10X Tris Wash Buffer
  - 225 mL deionized water

*Prepare MSD Blocker A Solution:*

- a) In a 50 mL tube combine (per plate):
  - 20 mL 1X Tris Wash Buffer
  - 600 mg MSD Blocker A (30 mg/mL or 3%)

*Prepare Antibody Dilution Buffer:*

- a) Prepare 10.5 mL per plate.
- b) In a 15 mL tube combine:
  - 3.5 mL MSD Blocker A Solution
  - 7 mL 1X Tris Wash Buffer
  - 105  $\mu$ L 10% MSD Blocker D-R

Begin with an MSD MULTI-SPOT Custom Plate. No pre-treatment is necessary.

### STEP 1

Add 150 $\mu$ L/well of MSD Blocker A Solution. Incubate at room temperature for 1 hour. Dilute A $\beta$ 1-40 peptide during this time.

## Notes:

*Read the entire detailed instructions before beginning work.*

*To avoid the possibility of aggregation of the standard peptide and/or sticking of the peptide to the dilution tubes, the dilutions should be prepared immediately before use.*

*The blocking step can be eliminated with no loss of sensitivity for peptides that are diluted in Tris Lysis Buffer. For more complex matrices, the importance of a blocking step must be empirically determined.*

*A larger amount of Tris Wash Buffer may be prepared at once and stored at room temperature for later use.*

*Solutions containing MSD Blocker A should be kept at 4°C and discarded after 14 days.*

*Save the plate packaging or copy the diagram of the capture antibody array into your notebook. Data will be labeled according to the location of each spot, not the actual name of the coating.*

*Prepare Peptide Solvent:*

- a) Prepare 1X peptide solvent by diluting 10X peptide solvent 10-fold.
- b) In a 15 mL tube combine:
  - 1 mL 10X peptide solvent
  - 9 mL distilled water
- c) Check the pH of the 1X peptide solvent using the provided pH test strips. Place one strip on a paper towel and pipette 100  $\mu$ L of the solution onto one strip. Do not dip the pH strip into the solvent. If the pH is lower than 9 (strip is brown), add 100  $\mu$ L of 10X peptide solvent, mix, and re-test with a new pH strip. Repeat until the pH is 9-10 (strip is blue).

*Prepare  $\alpha\beta$  peptide dilutions:*

- a) Check the label on the peptide vial/tube for the correct 1X peptide solvent addition volume in order to yield a 0.1 mg/mL stock.
- b) Vortex peptide to ensure that it is entirely dissolved.
- c) Prepare a dilution series of the peptide standard. Prepare the following dilutions in Tris Lysis Buffer (TLB):

*10  $\mu$ g/mL: 10  $\mu$ L of the 0.1 mg/mL stocks in 90  $\mu$ L of TLB  
0.1  $\mu$ g/mL: 5  $\mu$ L of the 10  $\mu$ g/mL solutions in 495  $\mu$ L of TLB  
10,000 pg/mL: 40  $\mu$ L of the 0.1  $\mu$ g/mL solution plus 360  $\mu$ L TLB  
3,160 pg/mL: 100  $\mu$ L of the 10,000 pg/mL solution plus 216  $\mu$ L TLB  
1,000 pg/mL: 100  $\mu$ L of the 3,160 pg/mL solution plus 216  $\mu$ L TLB  
316 pg/mL: 100  $\mu$ L of the 1,000 pg/mL solution plus 216  $\mu$ L TLB  
100 pg/mL: 100  $\mu$ L of the 316 pg/mL solution plus 216  $\mu$ L TLB  
32 pg/mL: 100  $\mu$ L of the 100 pg/mL solution plus 216  $\mu$ L TLB  
10 pg/mL: 100  $\mu$ L of the 32 pg/mL solution plus 216  $\mu$ L TLB  
3.2 pg/mL: 100  $\mu$ L of the 10 pg/mL solution plus 216  $\mu$ L TLB  
1 pg/mL: 100  $\mu$ L of the 3.2 pg/mL solution plus 216  $\mu$ L TLB  
0.32 pg/mL: 100  $\mu$ L of the 1 pg/mL solution plus 216  $\mu$ L TLB  
0.1 pg/mL: 100  $\mu$ L of the 0.32 pg/mL solution plus 216  $\mu$ L TLB  
0 pg/mL: Tris Lysis Buffer alone*

Wash plate four times with Tris Wash Buffer.

## STEP 2

Dispense 25  $\mu$ L/well of diluted peptide as shown in Figure 1 below. The remaining wells may be used for samples.

Incubate with shaking at room temperature for 1 hour. Prepare detection antibody during this time.

## Notes:

*The peptide solvent contains a low concentration of aqueous ammonia. The solutions and waste should be handled appropriately.*

*The 0.1 mg/mL peptide stock can be aliquotted and stored at -20  $^{\circ}$ C for up to one week in the silanized tubes provided. Refreezing, storing more dilute peptide, or storing for longer times are not recommended, as aggregation of the peptide may occur.*

*If the calibration curve is to be used to quantify the peptide in a complex matrix (culture supernatant, serum, CSF, etc.) you may wish to use a different diluent than Tris Lysis Buffer. For best results, MSD suggests a diluent that approximates the sample matrix.*

*This dilution scheme gives a "half-log" titration, in which data points will be evenly spaced when plotted on a log scale.*

Peptide Standard (pg/mL)												
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1	0.32	1	3.2	10	32	100	316	1000	3160	10,000
B	0	0.1	0.32	1	3.2	10	32	100	316	1000	3160	10,000
C	0	0.1	0.32	1	3.2	10	32	100	316	1000	3160	10,000
D	0											
E												
F												
G												
H												

**Notes:**

**Figure 1: Suggested plate layout for peptide standard.**

*Prepare biotinylated detection antibody:*

In a 15 mL tube combine:

- ❑ 27 µL biotinylated detection antibody
- ❑ 3600 µL Antibody Dilution Buffer

Wash plate four times with Tris Wash Buffer.

**STEP 3**

Add 25 µL/well of biotinylated detection antibody.

Incubate with shaking at room temperature for 1 hour. Prepare MSD SULFO-TAG streptavidin during this time.

*Prepare MSD SULFO-TAG streptavidin:*

In a 15 mL tube combine:

- ❑ 4.4 µL MSD SULFO-TAG streptavidin
- ❑ 5000 µL Antibody Dilution Buffer

**STEP 4**

Add 25 µL diluted MSD SULFO-TAG streptavidin to each well.

Incubate with shaking at room temperature for 1 hour. Prepare Read Buffer during this time.

*Dilute Read Buffer:*

In a 50 mL tube combine:

- ❑ 15 mL deionized water
- ❑ 5 mL 4X MSD Read Buffer T

Wash plates four times with Tris Wash Buffer.

**STEP 5**

Add 150 µL of diluted Read Buffer T, being careful to avoid bubbles in the wells.

Analyze with SECTOR™ Imager.

*Do not wash plate prior to the addition of the MSD SULFO-TAG streptavidin.*

*Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.*

*MSD Read Buffer T contains surfactant, which will easily form bubbles. If bubbles are present in the wells when imaged, the results will be inaccurate.*