

**MSD[®] Multiplexed β -amyloid Peptide Assays:
A β 40 and A β 42 - N-Terminal Detection
(6E10 Detection Antibody)
MULTI-SPOT[®] 4, 96-well Custom Plate**

Storage

MSD Catalog Items

- Read Buffer T(4X), with surfactant RT

R&D Custom Materials

- MULTI-SPOT 4, 96-well Custom plates 4°C
 - Tris Wash Buffer (10X) 4°C
 - SULFO-TAG[™] streptavidin 4°C
 - Biotinylated detection antibody 4°C
 - Blocker D-B (10%) 4°C
 - Blocker A (dry powder) 4°C
 - Peptide Solvent (10X) -20°C
 - A β 1-40 peptide (dry) -20°C
 - A β 1-42 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
- pH test strips
- Microcentrifuge tubes for making dilutions of peptide standards (silanized tubes are strongly recommended)
- Automated plate washer, Multidrop[®], or other efficient multi-channel pipetting equipment for washing 96 well plates
- Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 μ L and 150 μ 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 β -amyloid peptides. 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 peptides, 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 Blocker A solution, incubate 1 hour, wash.
2. Add peptides, 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 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 Blocker A Solution
 - 6.895 mL 1X Tris Wash Buffer
 - 105 μ L 10% Blocker D-B

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

STEP 1

Add 150 μ L/well of Blocker A.

Incubate at room temperature for 1 hour. Dilute A β 1-40 and A β 1-42 peptides during this time.

Notes:

Read the entire detailed instructions before beginning work.

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

A larger amount of Tris Wash Buffer

Prepare Peptide Solvent:

- a) Prepare 1X peptide solvent by diluting Peptide Solvent (10X) 10-fold.
- b) In a 15 mL tube combine:
 - 1 mL Peptide Solvent (10X)
 - 9 mL distilled water
- c) It is highly recommended that the pH of 1X peptide solvent be tested using a pH test strip. If the pH is lower than 9, add 100 μL of 10X peptide solvent, mix, and re-test with a new pH strip. Repeat until the pH is 9-10.

Prepare A β peptide dilutions:

- a) Check the labels on the peptide vials/tubes for the correct volume of 1X peptide solvent to add in order to yield a 0.1 mg/mL stock (each vial will be different).
- b) Vortex peptide to ensure that it is entirely dissolved.
- c) Prepare a dilution series of the two peptides. Prepare the following dilutions for the individual peptides in the appropriate diluent:

10 $\mu\text{g}/\text{mL}$: 10 μL of the 0.1 mg/mL stocks in 90 μL of diluent

0.1 $\mu\text{g}/\text{mL}$: 5 μL of the 10 $\mu\text{g}/\text{mL}$ solutions in 495 μL of diluent

Dilution series of combined peptides:

10,000 pg/mL: 40 μL each of the 0.1 $\mu\text{g}/\text{mL}$ solutions plus 320 μL diluent

3,160 pg/mL: 100 μL of the 10,000 pg/mL solution plus 216 μL diluent

1,000 pg/mL: 100 μL of the 3,160 pg/mL solution plus 216 μL diluent

316 pg/mL: 100 μL of the 1,000 pg/mL solution plus 216 μL diluent

100 pg/mL: 100 μL of the 316 pg/mL solution plus 216 μL diluent

32 pg/mL: 100 μL of the 100 pg/mL solution plus 216 μL diluent

10 pg/mL: 100 μL of the 32 pg/mL solution plus 216 μL diluent

3.2 pg/mL: 100 μL of the 10 pg/mL solution plus 216 μL diluent

1 pg/mL: 100 μL of the 3.2 pg/mL solution plus 216 μL diluent

0.32 pg/mL: 100 μL of the 1 pg/mL solution plus 216 μL diluent

0.1 pg/mL: 100 μL of the 0.32 pg/mL solution plus 216 μL diluent

0 pg/mL: diluent alone

Wash plate four times with Tris Wash Buffer.

STEP 2

Dispense 25 μL /well of diluted peptides or samples.

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

Prepare biotinylated detection antibody:

- a) Dilute biotinylated detection antibody to a final concentration of 10 nM.
- b) Use Antibody Dilution Buffer. Sufficient antibody is supplied to prepare 3 mL per plate.

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 stocks can be aliquotted and stored at -20 °C for up to one week. Silanized tubes are strongly recommended, to minimize peptide loss. Refreezing, storing more dilute peptides, or storing for longer times are not recommended, as aggregation of the peptides may occur.

Peptides can be diluted in 1% Blocker A in 1X Tris Wash Buffer. If the calibration curve is to be used to quantify peptides in a complex matrix (culture supernatant, serum, CSF, etc.) a different diluent may be desired

Wash plate four times with Tris Wash Buffer.

Notes:

STEP 3 Add 25 μ L/well of biotinylated detection antibody.

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

Prepare SULFO-TAG streptavidin:

- a) Dilute SULFO-TAG labeled streptavidin to a final concentration of 40 nM (2.2 μ g/mL).
- b) Use Antibody Dilution Buffer. Sufficient streptavidin is supplied to prepare 5 mL per plate.

STEP 4 Add 25 μ L diluted 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 Read Buffer T

Wash plates four times with Tris Wash Buffer.

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

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

STEP 5 Add 150 μ L/well of diluted Read Buffer T, being careful to avoid bubbles in the wells.

Analyze with SECTOR™ Imager.

Read Buffer T contains surfactant, which will easily form