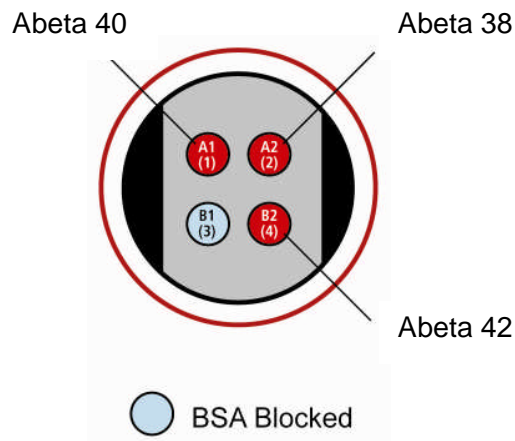


MSD[®] 96-Well MULTI-SPOT[®] Human/Rodent (4G8) Abeta Triplex Ultra-Sensitive Assay

Storage

Materials Included

<input type="checkbox"/> Read Buffer T (4X)	RT
<input type="checkbox"/> Blocker A (dry powder)	RT
<input type="checkbox"/> MULTI-SPOT Abeta Peptide 3-plex Plate	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] 4G8 Detection Antibody (50X) ¹	2-8°C
<input type="checkbox"/> Blocker G (100X)	2-8°C
<input type="checkbox"/> Dimethyl Sulfoxide (DMSO)	-20°C
<input type="checkbox"/> A β 1-38 Peptide (Lyophilized)	-20°C
<input type="checkbox"/> A β 1-40 Peptide (Lyophilized)	-20°C
<input type="checkbox"/> A β 1-42 Peptide (Lyophilized)	-20°C



The SECTOR Imager data file will identify spots according to their well location, not by the coated capture antibody name.

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.



Notes:

Other Materials & Equipment (not supplied)

- Deionized water for diluting Tris Wash Buffer and Read Buffer
- One 250 mL bottle
- Three 50 mL tubes
- Three 15 mL tubes
- Microcentrifuge tubes for making dilutions of peptide standards
- 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 5 μ L, 25 μ L and 150 μ L into a 96-well micro plate

Read the entire detailed instructions before beginning work.

Protocol at a Glance

This protocol takes approximately 3 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.

1. Add 150 μ L Blocker A to MSD plate, incubate for 1 hour, wash.
2. Add 25 μ L Detection Antibody Solution.
3. Add 25 μ L of Samples or Calibrator; incubate for 2 hours, wash.
4. Add 150 μ L 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

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

Prepare 1% Blocker A Solution:

- In a 50 mL tube combine:
- 50 mL 1X Tris Wash Buffer
 - 0.5 g Blocker A

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



Notes:

Prepare Detection Antibody Solution:

Prepare 3 mL per plate

- ❑ 60 μ L 50X SULFO-TAG 4G8 Detection Antibody
- ❑ 30 μ L 100X Blocker G
- ❑ 2910 μ L 1% Blocker A Solution

NOTE: For serum and plasma samples Blocker G should be omitted from the Detection Antibody Solution

Prepare 2X MSD Read Buffer T:

- a) Prepare 20 mL per plate
- b) In a 50 mL tube combine:
 - ❑ 10 mL deionized water
 - ❑ 10 mL 4X MSD Read Buffer T

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

Prepare A β 1-38, A β 1-40, and A β 1-42 peptide standards:

- a) Check the labels on the peptide vial/tubes for the correct volume of DMSO to dissolve the peptide vial in to yield a 0.1 mg/mL stock.
- b) Vortex the peptide solutions.
- c) Prepare the following dilution for each of the three peptides:
Prepare a 10 μ g/mL stock by adding 10 μ L of the 0.1 mg/mL stocks in 90 μ L of 1% Blocker A Solution.
Make a 0.1 μ g/mL stock of each peptide by adding 5 μ L of the 10 μ g/mL solution in 495 μ L of 1% Blocker A Solution.
- d) Prepare a dilution series of the combined peptides:
Prepare the highest Calibrator by adding 80 μ L of the 0.1 μ g/mL A β 40 peptide solution and 24 μ L each of the 0.1 μ g/mL A β 38 and A β 42 peptide solutions plus 672 μ L 1% Blocker A Solution.
Prepare the next Calibrator by transferring 200 μ L of the highest Calibrator to 400 μ L 1% Blocker A Solution. Mix well.
Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.

The 0.1 mg/mL peptide stocks can be aliquotted and stored at -20 °C for up to 6 weeks. MSD recommends making 20 aliquots of 25 μ L/tube. Refreezing, storing more dilute peptides, or storing for longer times is not recommended.

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

This yields the following Calibrator concentrations:

<u>Calibrator (pg/mL)</u>	<u>Aβ38</u>	<u>Aβ40</u>	<u>Aβ42</u>
Cal 7	3,000	10,000	3,000
Cal 6	1,000	3,333	1,000
Cal 5	333	1,111	333
Cal 4	111	370	111
Cal 3	37	123	37
Cal 2	12.3	41	12.3
Cal 1	4.1	13.7	4.1
0	0	0	0

It is recommended that both peptide standards and samples be assayed in duplicate.

Use 1% Blocker A Solution for Cal 0. These Calibrators will be sufficient to run an 8-point calibration curve in triplicate for multiple plates. Do not store diluted Calibrators.



Notes:

STEP 1 *Block Plate:*

- a) Add 150 μ L/well of 1% Blocker A Solution.
- b) Incubate at room temperature with shaking for 1 hour.
- c) Wash plate three times with 1X Tris Wash Buffer.

STEP 2 *Sample and Detection Antibody Addition:*

- a) Add 25 μ L/well of Detection Antibody Solution.
- b) Add 25 μ L/well of Samples or Calibrator.
- c) Incubate at room temperature with shaking for 2 hours.
- d) Wash plate three times with 1X Tris Wash Buffer.

CSF samples should be assayed 'neat' or no lower than a 1:2 dilution for optimal peptide sensitivity

STEP 3 *Read Plate:*

- a) Add 150 μ L/well of 2X MSD Read Buffer T.
- b) Read plate on Sector Imager immediately after Read Buffer addition and analyze data

Avoid bubbles while adding the Read Buffer; it will interfere with accurate reading of the plate.

