MSD® 96-Well MULTI-ARRAY®
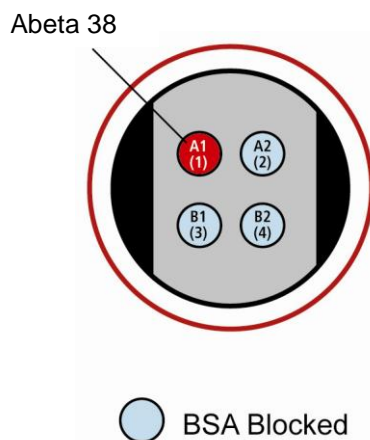# *Human/Rodent (4G8) Abeta 38 Ultra-Sensitive Assay*

Storage

Materials Included

❑ Read Buffer T (4X)   RT

❑ Blocker A (dry powder)   RT

❑ MULTI-SPOT® Abeta 38 Peptide Plate   2-8ºC

❑ Tris Wash Buffer (10X)   2-8ºC

❑ SULFO-TAG™ 4G8 Detection Antibody (50X)[1]   2-8ºC

❑ Dimethyl Sulfoxide (DMSO)   -20ºC

❑ Aβ1-38 peptide (Lyophilized)   -20ºC

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.

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 μL, 25 μL and 150 μL into a 96-well micro plate

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

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

b) In a 250 mL bottle combine:
   - ❑ 25 mL 10X Tris Wash Buffer
   - ❑ 225 mL deionized water

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 4ºC and discarded after 14 days.

Prepare Detection Antibody Solution:

Prepare 3 mL per plate
   - ❑ 60 μL 50X SULFO-TAG 4G8 Detection Antibody
   - ❑ 2940 μL 1% Blocker A 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

Prepare Aβ1-38 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 peptides to ensure that it is entirely dissolved.
c) Prepare the following dilution for the peptide:
   Prepare a 10 μg/mL stock by adding 10μL of the 0.1 mg/mL stocks in 90 μL of 1% Blocker A Solution.
   Make a 0.1μg/mL stock of peptide by adding 5μL of the 10 μg/mL solution in 495μL of 1% Blocker A Solution.
d) Prepare a dilution series of the peptide:
   Prepare the highest Calibrator by adding 24μL of the 0.1μg/mL Aβ38 peptide solution plus 776 μL 1% Blocker A Solution.
   Prepare the next Calibrator by transferring 200 μL of the highest Calibrator to 400 μL 1% Blocker A Solution. Mix well.
   Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.

   This yields the following Calibrator concentrations:

Calibrator (pg/mL)	Aβ38
Cal 7	3,000
Cal 6	1,000
Cal 5	333
Cal 4	111
Cal 3	37
Cal 2	12.3
Cal 1	4.1
0	0

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.

STEP 1   *Block Plate:*

     a)   Add 150 μL/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:*

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

     a)   Add 25 μL/well of Detection Antibody Solution.

     b)   Add 25 μL/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.

STEP 3   *Read Plate:*

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

     a)   Add 150 μL/well of 2X MSD Read Buffer T.

     b)   Read plate on Sector Imager immediately after Read Buffer addition and analyze data.