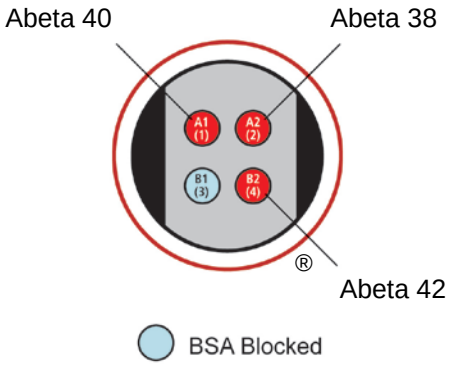
MSD® MULTI-SPOT Human (6E10) Abeta Triplex Assay

	Storage

Materials Included

- ❑ MULTI-SPOT® Abeta Peptide 3-Plex Plate — 2–8ºC
- ❑ SULFO-TAG™ 6E10 Detection Antibody (50X)[1] — 2–8ºC
- ❑ Aβ1-38 Peptide — ≤-70ºC
- ❑ Aβ1-40 Peptide — ≤-70ºC
- ❑ Aβ1-42 Peptide — ≤-70ºC
- ❑ Tris Wash Buffer (10X) — 2–8ºC
- ❑ Blocker G (100X) — 2–8ºC
- ❑ Blocker A (dry powder) — 2–8ºC
- ❑ Read Buffer T (4X) — RT



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

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 safety information is available in the product safety data sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

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

Other Materials & Equipment (not supplied)

Notes:

- ❑ Deionized water for diluting concentrated buffers
- ❑ 500 mL bottle
- ❑ 50 mL tubes
- ❑ 15 mL tubes
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (if desired)
- ❑ Automated plate washer or other efficient multi-channel pipetting equipment for washing 96-well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 25 µL and 150 µL into a 96-well microplate

Protocol at a Glance

Read the entire detailed instructions before beginning work.

1. Add Blocker A solution; incubate 1 hour; wash.
2. Add detection antibody.
3. Add samples or calibrators; incubate 2 hours; wash.
4. Add Read Buffer T and analyze plate.

This protocol can be completed in approximately 3 to 3 1/2 hours if each reagent is prepared during the preceding incubation. Alternatively, all reagents with the exception of diluted peptides can be prepared ahead of time. This lengthens the overall time required for the assay but frees up time during incubation steps.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer. 1X Tris Wash Buffer is used throughout the assay to dilute other reagents and wash plates. Approximately 350 mL per plate is required—more if using an automatic plate washer.

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

In a 500 mL bottle, combine:

- ❑ 35 mL 10X Tris Wash Buffer
- ❑ 315 mL deionized water

Prepare 1% Blocker A Solution. You will need 50 mL per plate.

Solutions containing Blocker A should be kept at 2–8ºC and discarded after 14 days.

In a 50 mL tube, combine:

- ❑ 50 mL 1X Tris Wash Buffer
- ❑ 500 mg Blocker A

Prepare Detection Antibody Solution. You will need 3.0 mL per plate at a 1X final concentration.

In a 15 mL tube, combine:

- ❑ 60 µL 50X SULFO-TAG 6E10 Detection Antibody
- ❑ 30 µL 100X Blocker G
- ❑ 2910 µL 1% Blocker A Solution

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

Notes:

Prepare Read Buffer T. You will need 20 mL per plate at a final 2X concentration.

In a 50 mL tube, combine:

- ❑ 10 mL 4X Read Buffer T
- ❑ 10 mL deionized water

Excess diluted read buffer may be stored in a tightly sealed container at room temperature.

Prepare Aβ1-38, Aβ1-40, and Aβ1-42 Peptide Standards. MSD supplies individual vials of Aβ peptide stock calibrators. For the lot-specific concentrations of each calibrator, refer to the certificate of analysis (C of A) supplied with the kit. You can also find a copy of the C of A at www.mesoscale.com.

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

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

Thorough mixing of stock and diluted kit reagents is required.

a) Thaw the stock calibrators at room temperature and mix well by vortexing.

b) Prepare individual diluted stocks of the Aβ1-38 and Aβ1-40 peptides as follows:
   - ❑ 20 µL of the supplied Aβ1-38 or Aβ1-40 stock calibrator
   - ❑ 180 µL of 1% Blocker A Solution.

   Mix well by vortexing.

Do not store diluted calibrators.

c) Combine the following to prepare the highest calibrator:
   - ❑ 22.5 µL of pre-diluted Aβ1-38 Peptide
   - ❑ 50 µL of pre-diluted Aβ1-40 Peptide
   - ❑ 15 µL of supplied Aβ1-42 Peptide
   - ❑ 212.5 µL of 1% Blocker A Solution

   Mix well by vortexing.

Both peptide standards and samples should be assayed in duplicate.

d) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 200 µL of 1% Blocker A Solution. Mix well by vortexing. Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.

e) Use 1% Blocker A Solution as the zero calibrator.

Begin with a MULTI-SPOT 96-well 4-Spot Abeta Peptide 3-Plex Plate. No pre-treatment is necessary.

Notes:

STEP 1

Add 150 µL/well of 1% Blocker A Solution.

Incubate with shaking at room temperature for 1 hour. During this time, prepare peptide standards and samples.

STEP 2

Wash plate(s) four times with at least 150 µL/well of 1X Tris Wash Buffer.

Add 25 µL/well of detection antibody solution.

Add 25 µL/well of the samples prepared during Step 1 incubation.

Incubate with shaking for 2 hours at room temperature.

CSF samples should be assayed 'neat' or diluted no more than 2-fold for optimal peptide sensitivity

Shaking the plate accelerates analyte capture.

STEP 3

Wash plate(s) four times with at least 150 µL/well of 1X Tris Wash Buffer

Add 150 µL/well of diluted Read Buffer T.

Analyze with MSD instrument.

Add read buffer carefully using reverse pipetting technique. Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.

Read plate(s) immediately after adding read buffer.

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The 6E10 antibody used in MSD Aβ assays is supplied by Covance Research Products, Inc
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