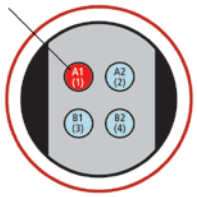
MSD® 96-Well MULTI-ARRAY® Human (6E10) Abeta 40 Ultra-Sensitive Kit

The first protocol has been optimized for quantifying Aβ 1-40 peptide in human cerebrospinal fluid (CSF).
The second protocol has been optimized for quantifying Aβ 1-40 peptide in human plasma samples.

	Storage
❑ Read Buffer T (4X)	RT
❑ Blocker A (dry powder)	RT
❑ MULTI-SPOT® 96-well, 4-Spot Human Abeta Peptide Plate(s)	2-8ºC
❑ Tris Wash Buffer (10X)	2-8ºC
❑ SULFO-TAG™ Anti-Aβ 40 Antibody (50X)	2-8ºC
❑ Dimethyl Sulfoxide (DMSO)	-20ºC
❑ Aβ1-40 peptide (Lyophilized)	-20ºC

6E10



BSA Blocked

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

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
- Two 50 mL tubes
- Two 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 25 µL and 150 µL into a 96-well micro plate

Protocol at a Glance

Read the entire detailed instructions before beginning work.

For other sample types, the second protocol should be used as a starting point for optimization of the assay in the chosen matrix.

The CSF protocol takes approximately 3 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.

1. Add Blocker A solution, incubate 30 minutes.
2. Add peptide or samples, incubate 1 hour, wash.
3. Add SULFO-TAG Detection Antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

Detailed instructions for use with Human Cerebrospinal Fluid (CSF) samples

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 10% Blocker A Solution:

a) In a 50 mL tube combine (per plate):
   - 20 mL 1X Tris Wash Buffer
   - 2 g Blocker A

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

Prepare Antibody Dilution Buffer:

a) Prepare 5 mL per plate.
b) In a 15 mL tube combine:
   - 500 µL 10% Blocker A Solution
   - 4.5 mL 1X Tris Wash Buffer

Prepare 1X Detection Antibody solution:

a) Prepare 3 mL of Detection Antibody per plate:
   - 60 µL 50X SULFO-TAG Anti-Aβ 40 Antibody
   - 2940 µL Antibody Dilution Buffer

Dilute Read Buffer:

a) Prepare 2X Read Buffer T solution.
b) In a 50 mL tube combine (per plate):
   - 10 mL deionized water
   - 10 mL 4X Read Buffer T

Prepare Aβ40 peptide dilutions:

a) Check the label on the peptide vial/tube 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 of the stock peptide:
   Prepare a 10 µg/mL stock by adding 10µL of the 0.1 mg/mL stocks in 90µL of 10% Blocker A Solution

   Make a 0.1µg/mL stock of each peptide by adding 5µL of the 10 µg/mL solution in 495µL of 10% Blocker A Solution

d) Prepare the assay dilution series:
   Prepare the highest Calibrator by adding 40 µL of the 0.1µg/mL Aβ40 peptide solution and 360µL 10% Blocker A Solution

   Prepare the next Calibrator by transferring 100 µL of the highest Calibrator to 300 µL 10% Blocker A Solution

   Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.

Notes:

Solutions containing Antibody Dilution Buffer should be kept at 4°C

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

The 0.1 mg/mL peptide stock can be aliquotted and stored at -20°C for up to 2 months. MSD recommends making 20 aliquots of 25 µL/tube. Refreezing, storing more dilute peptides, or storing for longer times are not recommended.

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.

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

Notes:

This yields the following Calibrator concentrations:

Calibrator (pg/mL)	Aβ40
Cal 7	10,000
Cal 6	2,500
Cal 5	625
Cal 4	156
Cal 3	39
Cal 2	9.8
Cal 1	2.4
0	0

Use 10% Blocker A Solution for Cal 0. These Calibrators will be sufficient to run an 8-point calibration curve for multiple plates. Do not store diluted Calibrators.

STEP 1 *Block Plate:*

a) Add 25µL/well of 10% Blocker A Solution.

b) Incubate at room temperature for 30 minutes with shaking.

c) **Do not wash plate before addition of standards or samples**

STEP 2 *Sample Addition:*

Abeta 40 peptide levels exhibit a broad dynamic range in CSF. Samples may need to be diluted for optimal peptide sensitivity.

Incubation may also be done at 4°C for 24 Hr with parallel assay performance

a) Dispense 25 µL/well of diluted peptide standards or samples.

b) Incubate with shaking at room temperature for 1 hour.

c) Wash plate three times with Tris Wash Buffer.

STEP 3 *Detection Antibody Addition:*

a) Add 25 µL/well of 1X SULFO-TAG Detection Antibody.

b) Incubate with shaking at room temperature for 1 hour.

c) Wash plates three times with Tris Wash Buffer.

STEP 4 *Read Plate:*

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

a) Add 150 µL/well of diluted 2X Read Buffer T, being careful to avoid bubbles in the wells.

b) Analyze with SECTOR Imager.

Detailed instructions for use with Human Plasma Samples

Notes:

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 3% 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%)

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

Prepare Antibody Dilution Buffer:

a) Prepare 10.5 mL per plate.

b) In a 15 mL tube combine:
- ❑ 3.5 mL 3% Blocker A Solution
- ❑ 7 mL 1X Tris Wash Buffer

Prepare 1X SULFO-TAG Detection Antibody:

b) Prepare 3 mL of Detection Antibody per plate:
- ❑ 60 µL 50X SULFO-TAG Anti Aβ 40 Antibody
- ❑ 2940 µL Antibody Dilution Buffer

Dilute Read Buffer:

c) Prepare 1X Read Buffer T solution.

d) In a 50 mL tube combine (per plate):
- ❑ 5 mL 4X Read Buffer T
- ❑ 15 mL deionized water

Prepare Aβ peptide dilutions:

a) Check the label on the peptide vial/tube 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 a dilution series of the peptide. Prepare the following dilutions in the appropriate diluent:

10 µg/mL: 10µL of the 0.1 mg/mL stock in 90 µL of diluent
0.1µg/mL: 5µL of the 10 µg/mL solution in 495µL of diluent
10,000 pg/mL: 40 µL of the 0.1µg/mL solution plus 360 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

STEP 1 *Block Plate:*

a) Add 150µL/well of 3% Blocker A Solution.
b) Incubate at room temperature for 1 hour with shaking.
c) Wash plate three times with Tris Wash Buffer.

STEP 2 *Sample Addition:*

a) Dispense 25 µL/well of diluted peptide standards or K_3EDTA plasma samples.
b) Incubate with shaking at room temperature for 1 hour.
c) Wash plate three times with Tris Wash Buffer.

STEP 3 *Detection Antibody Addition:*

a) Add 25 µL/well of 1X SULFO-TAG Detection Antibody.
b) Incubate with shaking at room temperature for 1 hour.
c) Wash plates three times with Tris Wash Buffer.

STEP 4 *Read Plate:*

a) Add 150 µL/well of diluted 1X Read Buffer T, being careful to avoid bubbles in the wells.
b) Analyze with SECTOR Imager.

Notes:

The 0.1 mg/mL peptide stock can be aliquotted and stored at -20 °C for up to 2 months. MSD recommends making 20 aliquots of 25 µL/tube. Refreezing, storing more dilute peptides, or storing for longer times are not recommended.

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

***Peptides should be diluted in 10% MSD Blocker A in 1X Tris Wash Buffer**. If the calibration curve is to be used to quantify peptide in human CSF, see "Detailed Instructions for use with human Cerebrospinal Fluid (CSF) samples".*

For other complex matrices (culture supernatant, serum, etc.) a different diluent may be desired. For best results, the peptide should be prepared in a diluent that mimics the sample matrix as closely as possible.

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