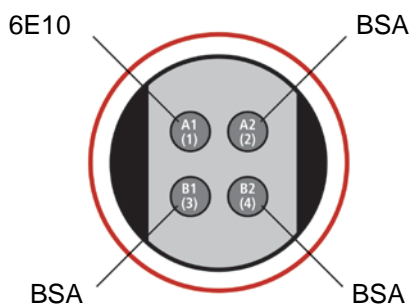


MSD[®] 96-Well MULTI-ARRAY[®] Human (6E10) Abeta 42 Ultra-Sensitive Kit

The first protocol has been optimized for quantifying A β 1-42 peptide in human cerebrospinal fluid (CSF).

An additional general protocol is also provided for optimization with other sample matrices.

	Storage
<input type="checkbox"/> Read Buffer T (4X)	RT
<input type="checkbox"/> Blocker A (dry powder)	RT
<input type="checkbox"/> MULTI-SPOT [®] 96-well, 4-Spot Human Abeta Peptide Plate(s)	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Anti A β 42 Antibody (50X)	2-8°C
<input type="checkbox"/> Dimethyl Sulfoxide (DMSO)	-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.

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



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 μ L and 150 μ L into a 96-well micro plate

Notes:

Read the entire detailed instructions before beginning work.

For other sample types, a general protocol is included at the end of the CSF protocol, which should be used as a starting point for optimization of the assay in the chosen matrix.

Protocol at a Glance

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.

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

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

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

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 10% Blocker A Solution:

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



Notes:

Prepare Antibody Dilution Buffer:

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

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

Prepare 1X Detection Antibody solution:

- a) Prepare 3 mL of Detection Antibody per plate:
 - ❑ 60 μ L 50X SULFO-TAG Anti A β 42 Antibody
 - ❑ 2940 μ L 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

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

Prepare A β 42 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.

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 μ L/tube. Refreezing, storing more dilute peptides, or storing for longer times are not recommended.

- c) Prepare the following dilution of the stock peptide:
Prepare a 10 μ g/mL stock by adding 10 μ L of the 0.1 mg/mL stocks in 90 μ L of 10% Blocker A Solution

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.

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 10% Blocker A Solution

- d) Prepare the assay dilution series:
Prepare the highest Calibrator by adding 10 μ L of the 0.1 μ g/mL A β 42 peptide solution and 323 μ L 10% Blocker A Solution

Prepare the next Calibrator by transferring 100 μ L of the highest Calibrator to 200 μ L 10% Blocker A Solution

Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.

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



Notes:

This yields the following Calibrator concentrations:

Calibrator (pg/mL)	Aβ42
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 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:

- Add 25 μ L/well of 10% Blocker A Solution.
- Incubate at room temperature for 30 minutes with shaking.
- Do not wash plate before addition of standards or samples**

STEP 2 Sample Addition:

- Dispense 25 μ L/well of diluted peptide standards or samples.
- Incubate with shaking at room temperature for 1 hour.
- Wash plate three times with Tris Wash Buffer.

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

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

STEP 3 Detection Antibody Addition:

- Add 25 μ L/well of 1X SULFO-TAG Detection Antibody.
- Incubate with shaking at room temperature for 1 hour.
- Wash plates three times with Tris Wash Buffer.

STEP 4 Read Plate:

- Add 150 μ L/well of diluted 2X Read Buffer T, being careful to avoid bubbles in the wells.
- Analyze with SECTOR™ Imager.

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



Notes:

General protocol for optimization with other sample matrices

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 4°C 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 42 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 A β 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 μ g/mL: 10 μ L of the 0.1 mg/mL stock in 90 μ L of diluent
0.1 μ g/mL: 5 μ L of the 10 μ g/mL solution in 495 μ L of diluent
10,000 pg/mL: 40 μ L of the 0.1 μ g/mL solution plus 360 μ L diluent
3,160 pg/mL: 100 μ L of the 10,000 pg/mL solution plus 216 μ L diluent
1,000 pg/mL: 100 μ L of the 3,160 pg/mL solution plus 216 μ L diluent
316 pg/mL: 100 μ L of the 1,000 pg/mL solution plus 216 μ L diluent
100 pg/mL: 100 μ L of the 316 pg/mL solution plus 216 μ L diluent
32 pg/mL: 100 μ L of the 100 pg/mL solution plus 216 μ L diluent
10 pg/mL: 100 μ L of the 32 pg/mL solution plus 216 μ L diluent
3.2 pg/mL: 100 μ L of the 10 pg/mL solution plus 216 μ L diluent
1 pg/mL: 100 μ L of the 3.2 pg/mL solution plus 216 μ L diluent
0.32 pg/mL: 100 μ L of the 1 pg/mL solution plus 216 μ L diluent
0.1 pg/mL: 100 μ L of the 0.32 pg/mL solution plus 216 μ L diluent
0 pg/mL: diluent alone

STEP 1 *Block Plate:*

- a) Add 150 μ L/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 μ L/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 μ L/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 μ L/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 μ L/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 can be diluted in 1% 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, plasma, 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.

