

MSD[®] 96-Well MULTI-ARRAY[®] sAPP α Assay

sAPP α is the extracellular protein that is released from the transmembrane amyloid precursor protein (APP) upon cleavage by α -secretase. Cleavage of APP by α -secretase precludes formation of A β peptides, as the cleavage site is within the A β sequence.

Storage

MSD Materials

<input type="checkbox"/> Read Buffer T, (with surfactant) (4X)	RT
<input type="checkbox"/> Blocker A	RT
<input type="checkbox"/> MULTI-SPOT [®] 96-well 4 Spot Custom plates	2-8°C
<input type="checkbox"/> Tris Wash Buffer (10X)	2-8°C
<input type="checkbox"/> SULFO-TAG [™] Anti-APP Antibody (50X)	2-8°C
<input type="checkbox"/> sAPP α Calibrator	≤ -70 °C

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and Read Buffer
- One 250 mL bottle
- Two 50 mL tubes
- Two 15 mL tubes
- Various microcentrifuge tubes for making serial dilutions of supernatants (if desired)
- 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

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



Protocol at a Glance

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to quantify sAPP α . The protocol takes approximately 3 to 3½ hours to complete if each reagent is prepared during the preceding incubation. All reagents can be prepared ahead of time. This lengthens the overall time required for the assay but frees up time during incubation steps.

Once desired results are achieved, the protocol can be streamlined to eliminate multiple incubation and wash steps to increase throughput.

1. Add blocking solution, incubate 1 hour, wash.
2. Add Calibrator and samples, incubate 1 hour, wash.
3. Add Detection Antibody, incubate 1 hour, wash.
4. Add Read Buffer and analyze plate.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer:

- a) 1X Tris Wash Buffer will be 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.
- b) In a 250 mL bottle combine:
 - 25 mL 10X Tris Wash Buffer
 - 225 mL deionized water

Prepare Blocking Solution-A:

- a) Prepare 20 mL per plate.
- b) In a 50 mL tube combine:
 - 20 mL 1X Tris Wash Buffer
 - 600 mg Blocker A (30 mg/mL or 3%)

Prepare Antibody Dilution Buffer:

- a) Prepare 3 mL per plate.
- b) In a 15 mL tube combine:
 - 1 mL Blocking Solution-A
 - 2 mL 1X Tris Wash Buffer

Begin with a MULTI-SPOT Custom plate. No pre-treatment is necessary.

STEP 1

Add 150 μ L/well of Blocking Solution-A.

Incubate at room temperature for 1 hour.

Notes:

Read the entire detailed instructions before beginning work.

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

Save the plate packaging or copy the diagram of the capture antibody array into your notebook. Data will be labeled according to the location of each spot, not the actual name of the coating.



Notes:

The sAPP α Calibrator can be diluted in a solution of 1% Blocker A in 1X Tris Wash Buffer. If the calibration curve will be used for quantification of proteins in a complex matrix (culture supernatant, serum, CSF, etc.) a different diluent may be desired.

The pH changes that occur in culture medium are detrimental to this assay, and it is recommended that culture medium samples be supplemented with HEPES buffer, pH 7.3 at a final concentration of 50 mM. Other matrices should be examined for pH effects, or also supplemented with HEPES buffer.

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

MSD recommends preparing the Calibrator dilutions in triplicate.

Prepare dilutions of sAPP α Calibrator:

1 μ g/mL: 6 μ L of the 50 μ g/mL solution plus 294 μ L of diluent
300 ng/mL: 100 μ L of the 1 μ g/mL solution plus 216 μ L diluent
100 ng/mL: 100 μ L of the 300 ng/mL solution plus 216 μ L diluent
30 ng/mL: 100 μ L of the 100 ng/mL solution plus 216 μ L diluent
10 ng/mL: 100 μ L of the 30 ng/mL solution plus 216 μ L diluent
3 ng/mL: 100 μ L of the 10 ng/mL solution plus 216 μ L diluent
1 ng/mL: 100 μ L of the 3 ng/mL solution plus 216 μ L diluent
300 pg/mL: 100 μ L of the 1 ng/mL solution plus 216 μ L diluent
100 pg/mL: 100 μ L of the 300 pg/mL solution plus 216 μ L diluent
30 pg/mL: 100 μ L of the 100 pg/mL solution plus 216 μ L diluent
10 pg/mL: 100 μ L of the 30 pg/mL solution plus 216 μ L diluent
0 pg/mL: diluent alone

Wash plates four times with Tris Wash Buffer.

STEP 2

Dispense 25 μ L/well of Calibrators and samples.

Incubate with shaking at room temperature for 1 hour. Prepare Detection Antibody during this time.

Prepare Detection Antibody:

- a) Prepare 3.0 mL per plate.
- b) In a 15 mL tube combine:
 - a. 2.94 mL cold Antibody Dilution Buffer
 - b. 60 μ L 50X Anti-APP Antibody
(Final concentration: 1X)

Wash plates four times with Tris Wash Buffer.

STEP 3

Add 25 μ L/well of Detection Antibody.

Incubate with shaking at room temperature for 1 hour. Prepare Read Buffer during this time.

Dilute Read Buffer:

In a 50 mL tube, combine (per plate):

- 5 mL 4X Read Buffer T (with surfactant)
- 15 mL deionized water

Wash plates four times with Tris Wash Buffer.

STEP 4

Add 150 μ L/well of diluted Read Buffer T.

INCUBATE PLATE AT ROOM TEMPERATURE (NO SHAKING) FOR 10 MINUTES

Analyze with SECTOR[®] Imager.

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

Note that bubbles in the Read Buffer will interfere with reliable imaging of the plate if carried into the wells.

The incubation in Read Buffer is essential for this assay.

The necessity of the incubation in Read Buffer may vary for different matrices.



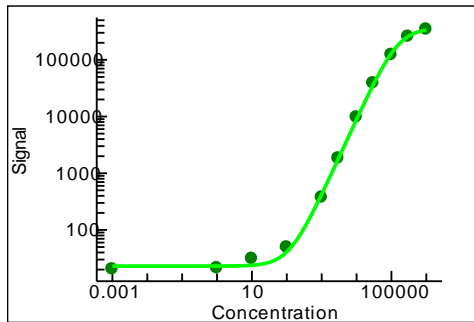
sAPP Calibrator

Recombinant Human sAPP α

Contents:	750 ng recombinant sAPP α protein
Concentration:	50 μ g/mL
Volume:	15 μ L
Preparation:	Recombinant human sAPP α protein was purified from overexpressing mammalian cells.
Storage:	Store at $\leq -70^{\circ}\text{C}$.
Quality Control:	Recombinant protein has been analyzed by SDS-PAGE and MSD MULTI-SPOT Assays.

MULTI-SPOT Assay Results

Typical titration curve for recombinant sAPP α using the MSD MULTI-SPOT sAPP α Assay.



Conc	Ave	StdDev	%CV	S/B
0	20	22	109	
1 pg/ml	32	8	27	2
10 pg/ml	47	6	14	2
100 pg/ml	74	3	4	4
300 pg/ml	92	11	11	5
1 ng/ml	373	16	4	18
3 ng/ml	1827	181	10	90
10 ng/ml	9711	423	4	478
30 ng/ml	38780	971	3	1907
100 ng/ml	122396	1162	1	6019
300 ng/ml	257081	8794	3	12643
1 ug/ml	344233	13166	4	16930

Detection limit (3 S.D. over background): 254 pg/ml

SDS-PAGE

A 0.5 mg sample of each sAPP protein was run on a 4-12% Bis-Tris NuPAGE gel to demonstrate purity (>95%).

