MSD 96-Well MULTI-ARRAY® Swedish sAPPβ Assay

Swedish sAPPβ is the extracellular protein that is released from the Swedish variant of the amyloid precursor protein (APP) upon cleavage by β-secretase. The Swedish variant contains a two amino acid substitution (at APP670 and 671), causing it to be more likely to undergo processing by β-secretase than the wild type protein, resulting in higher production of the amyloidogenic Aβ peptides.

MSD® Materials

	Storage
❑ Read Buffer T (4X)	RT
❑ Blocker A (dry powder)	RT
❑ MULTI-SPOT® 96-well 4-spot Swedish sAPPβ Plate(s)	2–8ºC
❑ Tris Wash Buffer (10X)	2–8ºC
❑ SULFO-TAG™ Anti-APP Detection Antibody (50X)[1]	2–8ºC
❑ Swedish sAPPβ Calibrator (50 µg/mL)	≤-70°C

Other Materials & Equipment (not supplied)

- ❑ 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 supernatants (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 micro plate

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

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

Protocol at a Glance

Notes:

1. Add blocking solution, incubate 1 hour, wash.
2. Add calibrator or samples, incubate 1 hour, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer T and analyze plate.

Read the entire detailed instructions before beginning work.

The following protocol is optimized for quantifying Swedish sAPPβ. The protocol takes approximately 3 to 3½ hours to complete. All reagents 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 at once and stored at room temperature for later use.

In a 500 mL bottle, combine:

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

Prepare 3% Blocker A Solution. You will need 20 mL per plate.

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

In a 50 mL tube, combine:

- ❑ 20 mL 1X Tris Wash Buffer
- ❑ 600 mg Blocker A (3% w/v)

Prepare Antibody Dilution Buffer. You will need 3 mL per plate.

In a 15 mL tube, combine:

- ❑ 2 mL 1X Tris Wash Buffer
- ❑ 1 mL of 3% Blocker A solution

Prepare Detection Antibody Solution. You will need 3 mL per plate.

Detection antibody solution should be stored in dark at 2–8ºC.

In a 15 mL tube, combine:

- ❑ 60 µL 50X SULFO-TAG Anti-APP Detection Antibody
- ❑ 2.94 mL cold Antibody Dilution Buffer

Prepare Read Buffer T. MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X. You will need 20 mL per plate at a 1X concentration.

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

In a 50 mL tube, combine:

- ❑ 15 mL deionized water
- ❑ 5 mL 4X Read Buffer T

Prepare Standards.

1000 ng/mL: 6µL of the 50 µg/mL stock solution plus 294 µL diluent
316 ng/mL: 100µL of the 1 µg/mL solution plus 216 µL diluent
100 ng/mL: 100 µL of the 300 ng/mL solution plus 216 µL diluent
32 ng/mL: 100 µL of the 100 ng/mL solution plus 216 µL diluent
10 ng/mL: 100 µL of the 30 ng/mL solution plus 216 µL diluent
3.2 ng/mL: 100 µL of the 10 ng/mL solution plus 216 µL diluent
1 ng/mL: 100 µL of the 3 ng/mL solution plus 216 µL diluent
0.32 ng/mL: 100 µL of the 1 ng/mL solution plus 216 µL diluent
0.10 ng/mL: 100 µL of the 300 pg/mL solution plus 216 µL diluent
0.032 ng/mL: 100 µL of the 100 pg/mL solution plus 216 µL diluent
0.010 ng/mL: 100 µL of the 30 pg/mL solution plus 216 µL diluent
0 ng/mL: diluent alone

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

STEP 1 Add Blocker A Solution

a) **Add** 150 µL/well of 3% Blocker A solution.
b) **Incubate** at room temperature with shaking for 1 hour.
c) **Wash** plate(s) three times with 300 µL/well of 1X Tris Wash Buffer.

STEP 2 Add Sample or Calibrator

a) **Add** 25 µL/well of samples or calibrator.
b) **Incubate** at room temperature with shaking for 1 hour.
c) **Wash** plate(s) three times with 300 µL/well of 1X Tris Wash Buffer.

STEP 3 Add Detection Antibody

a) **Add** 25 µL/well of detection antibody solution.
b) **Incubate** at room temperature with shaking for 1 hour.
c) **Wash** plate(s) three times with 300 µL/well of 1X Tris Wash Buffer.

STEP 4 Read Plate

a) **Add** 150 µL/well of 1X Read Buffer T.
b) **INCUBATE** PLATE AT ROOM TEMPERATURE (NO SHAKING) FOR 10 MINUTES
c) **Analyze** with SECTOR® Imager.

Notes:

The Swedish sAPP 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.

It is recommended that calibrators and samples be assayed in duplicate.

Shaking the plate accelerates analyte capture.

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.

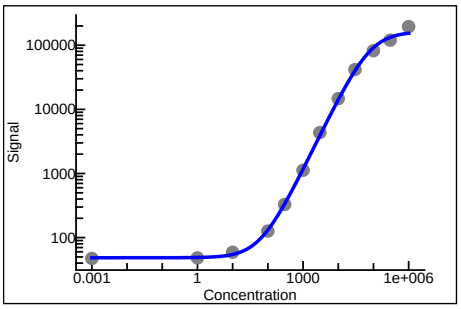
sAPP Calibrator

Recombinant Human Swedish sAPPβ

Contents: 750 ng recombinant Swedish sAPPβ protein
Concentration: 50 µg/mL
Volume: 15 µL
Preparation: Recombinant human sAPPβ protein containing the Swedish amino acid substitutions (670, 671) was purified from overexpressing mammalian cells.
Storage: Store at ≤-70°C.
Quality Control: Recombinant protein has been analyzed by SDS-PAGE and MSD MULTI-SPOT Assays.

MSD MULTI-SPOT Assay Results

Typical titration curve for recombinant Swedish sAPPβ using the MSD MULTI-SPOT Swedish sAPPβ assay.



Conc	Ave	StdDev	%CV	S/B
0	47	9	19	
0.01 ng/mL	48	11	24	1
0.03 ng/mL	59	22	37	1
0.1 ng/mL	126	18	14	3
0.3 ng/mL	329	15	4	7
1 ng/mL	1120	37	3	24
3.2 ng/mL	4345	176	4	92
10 ng/mL	14664	356	2	310
32 ng/mL	41785	125	0	883
100 ng/mL	81860	20236	25	1729
316 ng/mL	119720	3060	3	2529
1000 ng/mL	194794	19973	10	4115

Detection limit (3 S.D. over background): 39 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%).