MSD® 96-Well MULTI-SPOT sAPPα/sAPPβ Assay

Base Catalog No: K15120E

I. Materials Included

Reagent	Storage	Catalog No.	Size	Quantity Supplied		
				1-Plate Kit	5-Plate Kit	20-Plate Kit
MULTI-SPOT® 96-well 4-spot sAPPα/sAPPβ Plate	2–8 °C	N45120B-1	4-spot	1	5	20
APP Antibody (50X) (SULFO-TAG™ Detection Antibody)	2–8 °C	D21EA-2	1 vial	1	1	4
sAPPα Calibrator (50 µg/mL)	≤–70 °C	C01BS-2	1 vial	1	5	20
sAPPβ Calibrator (50 µg/mL)	≤–70 °C	C01BT-2	1 vial	1	5	20
Blocker A (dry powder)	RT	R93BA-4	15 g	1	1	1
Read Buffer T (4X)	RT	R92TC-3	50 mL	1	1	-
		R92TC-2	200 mL	-	-	1
Tris Wash Buffer (10X)	2–8 °C	R61TX-2	200 mL	1	1	-
		R61TX-1	1000 mL	-	-	1

RT = room temperature
Dash (-) = not applicable

II. 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
- Vortex mixer

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.

III. 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 the plate.

Read the entire detailed instructions before beginning work.

The following protocol is optimized for quantifying sAPPα and 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.

IV. 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 the 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 2–8 °C 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 the dark at 2–8 °C.

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:	6 µL of 50 µg/mL sAPPα Calibrator plus 6 µL of 50 µg/mL sAAPβ Calibrator solution plus 288 µL diluent
316 ng/mL:	100 µL of the 1000 ng/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 sAPPα/sAPPβ plate. No pre-treatment is necessary.

STEP 1 Add Blocker A Solution

a) **Add** 150 µL/well of 3% Blocker A solution to the plate(s).
b) **Incubate** the plate(s) at room temperature with shaking for 1 hour.
c) **Wash** the 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 to the plate(s).
b) **Incubate** the plate(s) at room temperature with shaking for 1 hour.
c) **Wash** the 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 to the plate(s).
b) **Incubate** the plate(s) at room temperature with shaking for 1 hour.
c) **Wash** the 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 to the plate(s).
b) **Incubate the plate(s) at room temperature** (NO SHAKING) for 10 minutes.
c) **Analyze** the plate(s) with a SECTOR® Imager instrument.

Notes:

The sAPP calibrators 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 a 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 also, supplemented with HEPES buffer as needed.

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

The sAPPα/sAPPβ assay is sensitive to the use of denaturing reagents and to the heat generated during sonification or homogenization.

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 Calibrators

Recombinant Human sAPPα and sAPPβ

Contents: 750 ng recombinant sAPPα and sAPPβ proteins

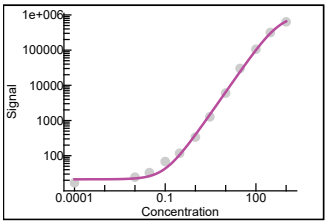
Concentration: 50 µg/mL
Volume: 15 µL
Preparation: Recombinant human sAPP proteins were purified from overexpressing mammalian cells.

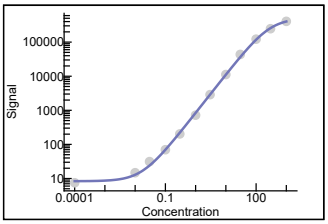
Storage: Store at ≤-70 °C.

Quality Control: Recombinant proteins have been analyzed by SDS-PAGE and MSD MULTI-SPOT Assays.

MSD MULTI-SPOT Assay Results

Typical titration curve for recombinant sAPP proteins using the MSD MULTI-SPOT sAPPα/β duplex assay.





Conc	Ave	StdDev	%CV	S/B	Ave	StdDev	%CV	S/B
0	17	10	62	1	10	6	62	1
0.01 ng/mL	32	9	28	2	20	6	31	2
0.03 ng/mL	33	7	22	2	31	5	16	4
0.1 ng/mL	68	8	12	4	70	14	20	9
0.3 ng/mL	118	7	6	7	203	18	9	26
1 ng/mL	337	3	1	20	720	60	8	93
3.2 ng/mL	1271	49	4	75	2878	219	8	371
10 ng/mL	6012	586	10	354	11193	1596	14	1444
32 ng/mL	30119	491	2	1772	43550	3390	8	5619
100 ng/mL	105764	17363	16	6221	121938	5558	5	15734
316 ng/mL	316441	6268	2	18614	246786	16891	7	31843
1000 ng/mL	634377	50042	8	37316	405610	35490	9	52337

Detection limits (3 S.D. over background)
sAPPα: 120 pg/ml
sAPPβ: 52 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%).