Spot the Difference™

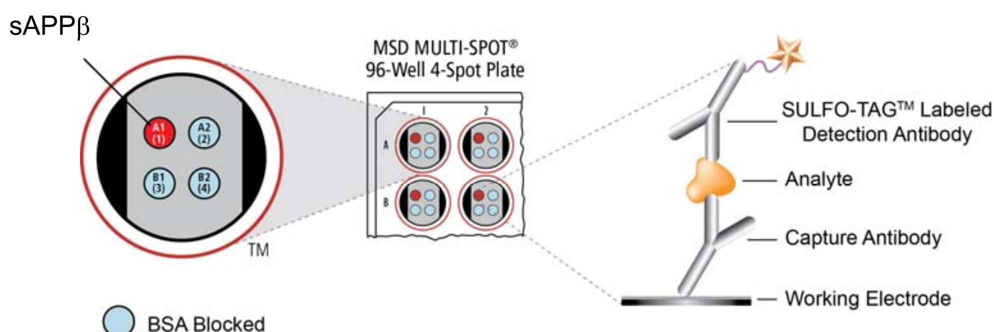
MSD® 96-Well MULTI-ARRAY Mouse/rat sAPPβ Assay

Separate protocols are provided and optimized for detection of sAPPβ in mouse and rat cerebrospinal fluid and mouse brain lysates.

Storage

MSD Materials

❑	Read Buffer T (4X)	RT
❑	Blocker A	RT
❑	SDS (10%)	RT
❑	NP-40	RT
❑	MULTI-SPOT® 96-well 4-Spot sAPPβ plate(s)	2-8ºC
❑	SULFO-TAG™ Anti-APP (22C11) Antibody (50X)[1]	2-8ºC
❑	Tris Wash Buffer (10X)	2-8°C
❑	Tris Lysis Buffer (1X)	2-8°C
❑	Phosphatase Inhibitor I (100X)	2-8°C
❑	Phosphatase Inhibitor II (100X)	2-8°C
❑	DTT (1M)	-80ºC
❑	Protease Inhibitor Solution (50X)	≤-10ºC
❑	Diluent 7	≤-10ºC
❑	sAPPβ Calibrator (50 μg/mL)	≤-70ºC



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

[1] Some SULFO-TAG conjugated detection antibodies may be light-sensitive, so they should be stored in the dark.

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

Notes:

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Tris Wash Buffer (10X) and Read Buffer T (4X)
- ❑ One 250 mL bottle
- ❑ Two 50 mL tubes
- ❑ One 15 mL tube
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Various microcentrifuge tubes for making serial dilutions of lysates (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-well plate

Read the entire detailed instructions before beginning work.

CSF Protocol at a Glance

The following protocol is optimized for quantifying sAPPβ in rodent cerebrospinal fluid. The protocol can be completed in approximately 3 to 3½ hours if each reagent is prepared during the preceding blocking or incubation step.

1. Add blocking solution, incubate 1 hour, wash.
2. Add 25 µL of denaturing assay solution.
   Add 25 µL of samples or calibrator, incubate 1 hour, wash.
3. Add 25 µL of detection antibody, incubate 1 hour, wash.
4. Add 150 µL of Read Buffer T 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

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

Prepare Blocker A Solution:

a) Prepare 20 mL per plate.

b) In a 50 mL tube combine:
   - ❑ 20 mL 1X Tris Wash Buffer
   - ❑ 600 mg Blocker A (3% w/v)

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

Prepare Denaturing Assay Solution:

a) Prepare 6 mL per plate.
b) In a 15 mL tube combine:
- ❑ 5730 µL Diluent 7
- ❑ 120 µL 10% SDS
- ❑ 120 µL NP-40
- ❑ 30 µL 1M DTT

Notes:

Denaturing assay solution should be made immediately prior to use, kept at 2-8°C and discarded after use.

Prepare Antibody Dilution Buffer:

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

Prepare Recombinant sAPPβ Calibrators:

a) Prepare highest calibrator by adding 10 µL of recombinant sAPPβ (50 µg/mL) to 490 µL of denaturing assay solution.
b) Prepare the next calibrator by transferring 100 µL of the highest calibrator to 200 µL of denaturing assay solution.
c) Repeat 3-fold serial dilutions 5 additional times to generate 7 calibrators.
d) This yields the following calibrator concentrations:

Calibrator	sAPPβ (ng/mL)
Cal 7	1000
Cal 6	333
Cal 5	111
Cal 4	37
Cal 3	12
Cal 2	4.1
Cal 1	1.3
Cal 0	0

e) Use denaturing assay solution for Cal 0. These calibrators will be sufficient to run an 8-point calibration curve in triplicate for one plate. Do not store diluted calibrators.

Calibrator dilutions should be kept at 2-8°C and discarded after use.

sAPPβ 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, plasma, etc.) a different diluent may be desired.

Prepare the 1X Detection Antibody Solution:

a) Prepare 3.0 mL per plate:
b) In a 15 mL tube combine:
- ❑ 2.94 mL cold Antibody Dilution Buffer
- ❑ 60 µL 50X Anti-APP (22C11) Antibody

(Final concentration: 1X)

Dilute Read Buffer T:

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

(Final concentration: 2X)

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

Begin with a MULTI-SPOT 96-well 4-Spot sAPPβ Plate.
No pre-treatment is necessary.

Notes:

STEP 1 ***Block Plate:***

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

STEP 2 ***Start the Incubation:***

a) Add 25 µL/well of denaturing assay solution.
b) Immediately add 25 µL/well of calibrator or sample.
c) Incubate with shaking for 1 hour at room temperature.
d) Wash plate three times with 1X Tris Wash Buffer.

Shaking a 96-well MSD MULTI-ARRAY® or MULTI-SPOT plate accelerates capture at the working electrode.

STEP 3 ***Detection Antibody Addition:***

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

STEP 4 ***Read Plate:***

a) Add 150 µL/well of 2X Read Buffer T.
b) Read plate on SECTOR Imager immediately after read buffer addition and analyze data.

Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.

Plates can be imaged immediately following the addition of read buffer.

Brain Protocol at a Glance

The following protocol is optimized for quantifying sAPPβ in mouse brain lysates. The protocol can be completed in approximately 3 to 3½ hours if each reagent is prepared during the preceding incubation. All reagents with the exception of diluted lysates can also 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 lysate, incubate 1 hour at room temperature, wash.
3. Add detection antibody, incubate 1 hour, wash.
4. Add Read Buffer T 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 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 Blocker A Solution:

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

Prepare Antibody Dilution Buffer:

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

Notes:

Read the entire detailed instructions before beginning work.

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

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

Notes:

Begin with a MULTI-SPOT 96-well 4-Spot sAPPβ Plate.
No pre-treatment is necessary.

STEP 1

Add 150 µL/well of Blocker A Solution.

Plates may also be blocked overnight at 2-8ºC.

Incubate with shaking at room temperature for 1 hour. Prepare Complete Tris Lysis Buffer, denaturing assay solution, and dilute brain lysates during this time.

Prepare Complete Tris Lysis Buffer:

a) It is important that all of the reagents (including the Tris Lysis Buffer) are at room temperature before mixing. To 10 mL of Tris Lysis Buffer, add the following:
   - ❑ 100 µL Phosphatase Inhibitor I (100X stock)
   - ❑ 100 µL Phosphatase Inhibitor II (100X stock)
   - ❑ 200 µL Protease Inhibitor Solution (50X stock)

b) Keep Complete Tris Lysis Buffer on ice until use.

Complete Tris Lysis Buffer should be ice cold before use.

Complete Tris Lysis Buffer and denaturation assay solution should be made immediately prior to use.

Prepare Denaturing Assay Solution:

a) In a 15 mL tube combine:
   - ❑ 60 µL 10% SDS
   - ❑ 60 µL NP-40
   - ❑ 15 µL 1M DTT
   - ❑ 2865 µL Complete Tris Lysis Buffer

Prepare samples or positive and negative brain lysates:

(Note: Recommendations for brain lysate handling are provided. However, the suggested concentrations listed below may need to be adjusted depending upon specific samples tested.)

a) Thaw brain lysate samples on ice, and dilute immediately before use. Keep on ice during all manipulations, and discard all remaining thawed unused material.
b) Dilute lysates in denaturing assay solution to prepare a sample containing 2 µg/µL of lysate (50 µg/well in 25 µL).
c) Additional dilutions may be prepared from the 2 µg/µL lysate sample if desired. Use the denaturing assay solution for any further lysate dilutions.

Wash plates three times with 1X Tris Wash Buffer.

STEP 2

Add 25 µL/well of diluted brain lysates.

Shaking a 96-well MSD MULTI-ARRAY or MULTI-SPOT plate accelerates capture at the working electrode.

Incubate with shaking for 1 hour at room temperature. Prepare detection antibody solution during this time.

Notes:

Prepare Detection Antibody Solution:

a) Prepare 3.0 mL per plate.
b) In a 15 mL tube combine:
- ❑ 2.94 mL cold Antibody Dilution Buffer
- ❑ 60 µL 50X Anti-APP (22C11) Antibody (Final concentration: 1X)

Wash plates three times with 1X Tris Wash Buffer.

STEP 3 Add 25 µL/well of detection antibody solution.

Incubate with shaking at room temperature for 1 hour. Prepare 2X Read Buffer during this time.

Dilute Read BufferT:

In a 50 mL tube, combine (per plate):
- ❑ 10 mL 4X Read Buffer T
- ❑ 10 mL deionized water (Final concentration: 2X)

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

Wash plates three times with 1X Tris Wash Buffer.

Bubbles in the read buffer will interfere with reliable imaging of the plate if carried into the wells.

STEP 4 Add 150 µL/well of 2X Read Buffer T.

Analyze with SECTOR Imager plate reader.

Plates can be imaged immediately following the addition of read buffer.

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