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

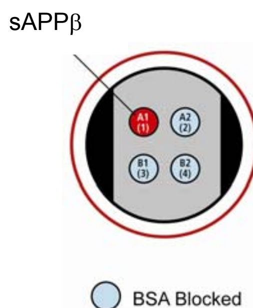
This protocol has been optimized for detection of soluble amyloid precursor proteinβ in mouse and rat cerebrospinal fluid.

	Storage

MSD Materials

❑ Read Buffer T (with surfactant), 4X	RT
❑ Blocker A	RT
❑ 10% Sodium Dodecyl Sulfate (SDS)	RT
❑ Nonidet P-40 (NP-40)	RT
❑ MULTI-SPOT® 96-well 4 Spot sAPPβ plate(s)	2-8ºC
❑ Tris Wash Buffer (10X)	2-8°C
❑ SULFO-TAG™ Anti-APP (22C11) Antibody (50X)	2-8ºC
❑ 1M Dithiothreitol (DTT)	-20ºC
❑ GF1 Assay Diluent	-20ºC
❑ sAPPβ Calibrator (50 μg/mL)	≤-70 °C

sAPPβ



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.

Notes:

Other Materials & Equipment (not supplied)

- Deionized water for diluting Wash Buffer and Read Buffer
- 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 10 – 150 µL into a 96-well micro plate
- Microcentrifuge tubes (e.g. VWR catalog# 20170-662)
- Adhesive Plate Seals

Protocol at a Glance

Read the entire detailed instructions before beginning work.

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.

Step 1. Add Blocking solution, incubate 1 hour, wash.

Step 2. Add 25 µL of Denaturing Assay Solution
Add 25 µL of samples or Calibrator, incubate 1 hour, wash.

Step 3. Add 25 µL of Detection Antibody, incubate 1 hour, wash

Step 4. Add 150 µL of Read Buffer and analyze plate.

Detailed Instructions

Prepare a stock of 1X Tris Wash Buffer:

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

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 Blocker A Solution:

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

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

Notes:

Prepare Denaturing Assay Solution:

Denaturing Assay Solution should be made immediately prior to use, kept at 4°C and discarded after use.

a) Prepare 6 mL per plate.
b) In a 15 mL tube combine:
   - ❑ 5730 µL GF1 Assay Diluent
   - ❑ 120 µL 10% SDS
   - ❑ 120 µL NP-40
   - ❑ 30 µL 1M DTT

Prepare Antibody Dilution Buffer:

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

Prepare sAPPβ Calibrators:

Calibrator dilutions should be kept at 4°C and discarded after use.

a) Prepare highest Calibrator by adding 10 µL of 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	1,000
Cal 6	333
Cal 5	111
Cal 4	37
Cal 3	12
Cal 2	4.1
Cal 1	1.3
Cal 0	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.

Prepare the 1X Detection Antibody Solution:

Notes:

a) In a 15 mL tube combine:
   - ❑ 60 µL of 50X SULFO-TAG Anti-APP (22C11) Antibody
   - ❑ 2940 µL of Antibody Dilution Buffer

b) This will yield 3 mL of diluted Detection Antibody Solution at the working concentration with sufficient volume for one plate.

Dilute Read Buffer:

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

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

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.

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.

Avoid bubbles while adding the Read Buffer; it will interfere with accurate reading of the plate.