Ultrasensitive detection of total and phosphorylated Tau protein in human blood using SMC® technology

Raad Gitan, Matthew Lippold, Jared Fiske, Rick Wiese

Introduction

Few analytes epitomize the utility of ultrasensitive immunoassays in blood-based biomarker research better than total and phosphorylated Tau protein. Tau protein is released from diseased and damaged neurons into cerebrospinal fluid (CSF) as well as peripheral circulation (at lower levels). Although Tau is not the sole biomarker for any individual neurodegenerative disease, it is widely used to support the identification and qualification of Alzheimer's Disease (AD) in association with other biomarkers or brain scans. The ability of researchers to measure this analyte at low abundance enables novel studies that address the role of this marker from health to disease, paving the way for future therapies aimed at slowing or even halting disease progression prior to late-stage symptoms.

The importance of total and phosphorylated Tau for those studying both healthy and aberrant brain biology necessitates the availability of ultrasensitive immunoassays capable of accurate and reliable measurements, especially at low abundance in blood samples. We have harnessed the state-of-the-art SMCxPRO® immunoassay system to develop the SMC® total Tau and phosphorylated Tau (pT181, pT217 and pT231) high sensitivity assays for analysis of human serum, plasma, and CSF matrices. SMC® technology delivers ultrasensitive biomarker measurements by combining rigorous assay development processes with a robust and easy-to-use platform, overcoming the limits of traditional sandwich ELISA assays in low-abundant biomarker studies.

This suite of total and phosphorylated Tau assays demonstrates excellent performance characteristics for spike/recovery values and dilutional linearity (80-120% for all three matrices). Total and phosphorylated Tau protein was detected in all sample types tested. Increased total and phosphorylated Tau sample levels in AD samples compared to normal serum, plasma, and CSF samples was also observed.

Developing therapeutics for diseases such as AD and multiple sclerosis (MS) requires the completion of stringent preclinical and clinical trials. Our menu of SMC® assays ensures researchers can successfully apply our kits towards generating accurate and reproducible data needed to qualify biomarkers as targets for novel treatments. The new phosphorylated (pT217 and pT231) Tau protein assays join our portfolio of highly verified SMC® biomarker kits as we aim to support the current and future needs in neuroscience biomarker research.

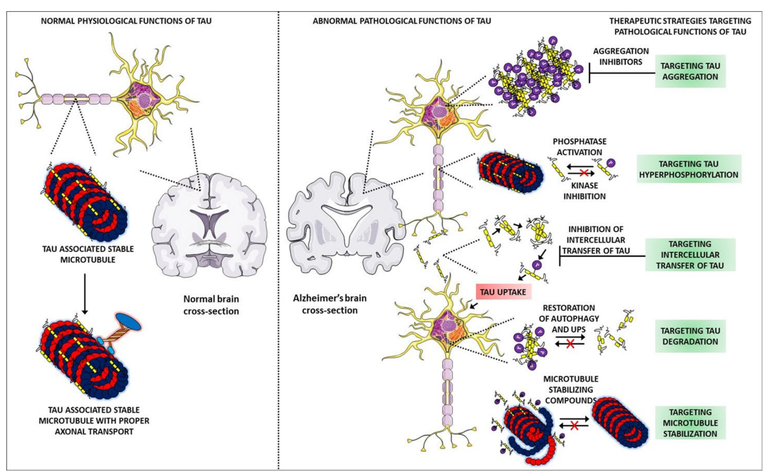
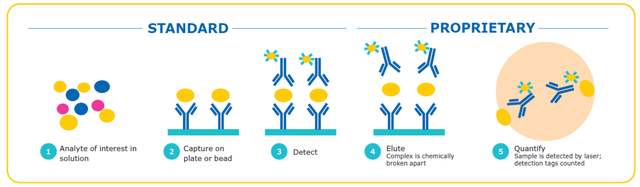


Figure 1. Tau normal physiological and pathological functions. Role of tau protein in Alzheimer's disease: The prime pathological player. S Muralinder, S Ambi, S Sekaran, D Thirumalai, B Palaniappan. Int J of Biol Macromolecules. 163:1559-1617. 2020. https://www.sciencedirect.com/science/article/pii/S0141813020340861

Methods

SMC® Technology

SMC® technology provides maximum immunoassay performance while following a workflow similar to traditional ELISA technology, as shown below. By combining a unique assay elution step and robust digital counting, SMC® technology achieves improved signal-to-noise ratios over traditional immunoassay technologies. The SMC® technology thus provides enhanced quantification at both low and high levels of expression on one complete system.



Steps 2-3
Antibodies translate each biomarker into a signal

Step 4
Fluorescent dye-labeled detection antibodies dissociate from the immunocomplex

Step 5
On the SMCxPRO® instrument, signal is collected from free-floating fluorochromes using a rotating laser, camera, and counting individual molecules by an Avalanche Photodiode

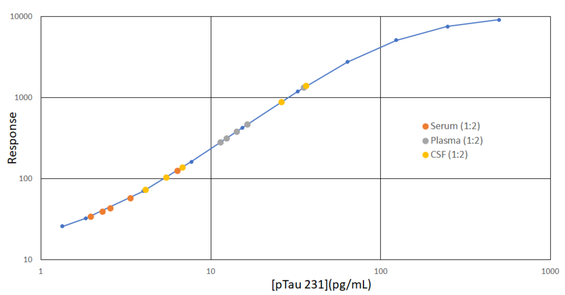
Benefits of SMC® Immunoassays for Neuroscience Research

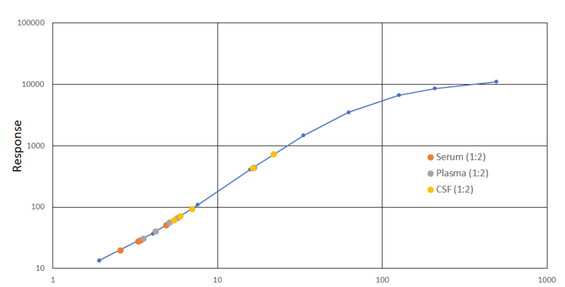
- **Empowers study of low-abundant neurodegenerative biomarkers in blood-based compartments**
- **Perform key experiments while conserving precious samples through dilution**
- **Overcome quantitation challenges minimizing sample matrix interference while maximizing specific signal**
- **Progress neurodegenerative disease studies by enabling profiling markers from health to disease, and from youth to old age**

Figure 2. SMC® immunoassay technology enables accurate measurement of low-abundant biomarkers in biological samples through a proprietary assay mechanism involving the interrogation of individual molecular binding events. Coupled with the SMCxPRO® ultrasensitive immunoassay system, researchers are empowered to confidently study key neurodegenerative markers, including those in blood-based biomarker studies.

Results

Figure 3. SMC® pTau 217 and pTau 231 assay sample response values are illustrated within the context of the standard curves generated for each assay. Five samples each of normal human serum, plasma and cerebrospinal fluid (CSF) were assayed at a 1:2 dilution and plotted.





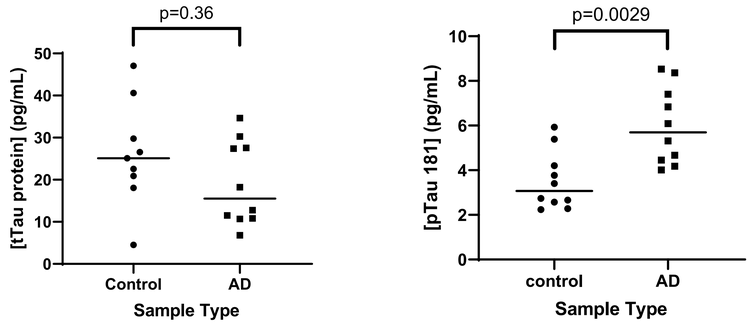


Figure 4. Scatter plots representing data obtained utilizing the newly developed pTau 217 and pTau 231 kits as well as currently available total Tau and pTau 181 kits. Each graph contains the values of 10 Alzheimer's Disease plasma samples (AD) and 10 control plasma samples (clinically normal). P values are displayed for each graph and represent the results of the Mann-Whitney test.

	Control Samples									
Analyte	1	2	3	4	5	6	7	8	9	10
total Tau (pg/mL)	47.1	BLOQ	29.8	25.1	4.5	22.6	40.6	20.9	18.1	26.6
pTau 181 (pg/mL)	2.7	2.2	5.9	2.3	3.4	5.4	4.2	2.6	2.7	3.8
pTau 217 (pg/mL)	11.1	5.3	20.2	20.2	31.8	28.9	30.2	9.6	21.7	18.8
pTau 231 (pg/mL)	21.4	6.0	83.4	31.1	15.9	16.1	61.4	13.6	35.2	63.5
	Alzheimers Disease Samples									
Analyte	1	2	3	4	5	6	7	8	9	10
total Tau (pg/mL)	34.7	27.4	30.2	12.8	6.8	10.7	18.2	10.8	27.6	11.5
pTau 181 (pg/mL)	7.4	8.5	8.4	4.2	4.4	6.8	4.0	4.7	5.3	6.1
pTau 217 (pg/mL)	25.3	20.8	23.5	23.3	19.5	21.8	26.5	21.6	33.0	29.0
pTau 231 (pg/mL)	134.1	144.0	189.5	111.1	61.9	111.7	135.7	113.4	104.4	126.4

Table 1. Data obtained from the Tau protein assays is displayed in Table 1. Displayed data is the average of three replicate measurements for each of the indicated plasma samples. Phosphorylated and Total Tau values are calculated against kit specific synthetic standards which are not cross calibrated across the individual kits, resulting in phosphorylated values greater than total at times. Note: Sample identification numbers have no relationship between Control and Alzheimer's Disease samples.

SMC® Neuroscience Biomarker Kits (kit catalog number)			
α-Synuclein (03-0196-00)	p-α-Synuclein (S129) (03-0188-00)	Amyloid Beta 1-40 (03-0145-00)	Amyloid Beta 1-42 (03-0146-00)
BDNF (03-0171-00)	GFAP (03-0203-00)	NPTX2 (03-0199-00)	SNAP-25 (03-0206-00)
TDP-43 (03-0205-00)	UCHL1 (03-0183-00)	NF-L (03-0202-00)	Total Tau (03-0185-00)
p-Tau T181 (03-0184-00)	p-Tau T217 available soon	p-Tau T231 available soon	

Table 2. List of highly-verified neuroscience biomarker kits for use with the state-of-the-art SMCxPRO® Ultrasensitive Immunoassay System. Kit catalog numbers listed in parentheses. For Research Use Only. Not For Use In Diagnostic Procedures.

Explore more at SigmaAldrich.com/smc-neuro