Abstract # 2453

ECL-based immunoassay for markers of neurodegeneration in rodent models

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1 Abstract

Animal models of neurodegenerative disease (ND) are a critical component of mechanistic, diagnostic, and therapeutic research. Monitoring biomarkers is key to recapitulating human disease pathology in research. Large quantities of tissue samples cannot be collected from small animals such as mice and rats, thus the ability to multiplex measurements with a small sample volume is advantageous. Here, we demonstrate both that a multiplexed assay for human glial fibrillary acidic protein (GFAP), neurofilament light (NF-L), and total Tau (Tau) cross-reacts with the mouse and rat analogues of these biomarkers and that the assays are suitable for measuring mouse and rat samples ranging from healthy controls to various models of ND.

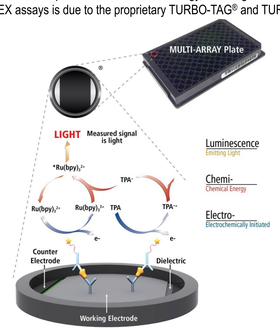
The MSD S-PLEX Neurology Panel 1 kit uses ultrasensitive electrochemiluminescence immunoassay technology to measure GFAP, NF-L, and Tau simultaneously in a 96-well plate format with standard liquid handling techniques. Serum and plasma samples from healthy mice and rats (n=5 each) were tested up to a 5-fold dilution, and brain lysates were tested from 0.032-4 µg/mL of total brain protein. Common commercially sourced mouse models of Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's disease were tested to determine detectability of GFAP, NF-L, and Tau.

The quantifiable range was 0.26-880 pg/mL for GFAP, 1.19-4,330 pg/mL for NF-L and 0.04-153 pg/mL for Tau based on calibration with the human proteins. GFAP, NF-L, and Tau were detectable in all serum and plasma samples after a 2-fold dilution, requiring only 12.5µL of neat sample per determination (for all three markers). All analytes were detectable in mouse and rat brain lysates tested at 0.160 µg/mL of total brain protein, and GFAP and NF-L were further detectable in lysates tested at 0.032 µg/mL. Linear dilution was observed in serum samples for all analytes in mice and for GFAP and Tau in rats, with average recoveries of 92-118% for 5-fold dilutions. In brain lysates, all analytes diluted linearly from 20 to 0.160 µg/mL of total brain protein, with average recovery of 88% for GFAP, 86% for NF-L, and 92% for Tau. We measured biomarkers in commercially sourced serum, plasma, cerebrospinal fluid, brain, and spinal cord from mouse ND models, including tauopathy models expressing human tau transgenes. GFAP, NF-L, and Tau were found in varying amounts dependent on the mouse line.

Mouse and rat cross-reactivity with the ultrasensitive multiplexed human panel is sufficient to measure GFAP, NF-L, and Tau in healthy mouse and rat samples, and in samples from several popular ND mouse models. This provides an advanced tool for research in the ever-expanding menu of rodent neurodegeneration models.

2 Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates. The MSD® S-PLEX Neurology Panel 1 is a three-analyte ultrasensitive panel. The S-PLEX® platform uses ECL technology, retaining its well-known advantages and superior analytical performance. The improved sensitivity of S-PLEX assays is due to the proprietary TURBO-TAG® and TURBO-BOOST® reagents.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

Step 1: Assemble
a. Coat plate with Biotin Antibody
b. Add samples and calibrator
c. Add TURBO-BOOST Antibody Solution

Step 2: Enhance
a. Add Enhance Solution
b. Add TURBO-TAG Detection Solution

Step 3: Read
a. Add Read Buffer
b. Read plate

3 Tested Samples

Table 1: Mouse and Rat Sample Descriptions

Species	Strain	Matrix	Age, Gender	Description
Mouse	C57BL/6	K2EDTA Plasma	Unspecified	Pooled plasma from C57BL/6 mice, gender unspecified
Mouse	ARTE10	K2EDTA Plasma	6 months, F	AD model expressing human APP (Swedish mutation) and Presenilin 1
Mouse	1349	K2EDTA Plasma	6 months, F	AD model expressing human APP (Swedish mutation)
Mouse	34711	K2EDTA Plasma	10 weeks, M	Homozygous AD model expressing human APP (Swedish, Arctic, and Austrian mutations)
Mouse		CSF		
Mouse	12836	K2EDTA Plasma	8 weeks, M	Hemizygous ALS model expressing human TDP-43
Mouse		CSF		
Mouse	BALB/C	K2EDTA Plasma	Unspecified	Pooled plasma from BALB/C mice
Mouse	BALB/C	Serum	Unspecified	Pooled serum from BALB/C mice
Mouse	C57BL/6	Whole Brain Lysate	Unspecified	Mouse Brain Whole Tissue Lysate
Rat	Sprague-Dawley	K2EDTA Plasma	Unspecified	Pooled plasma from Sprague Dawley rats
Rat	Sprague-Dawley	Serum	Unspecified	Pooled serum from Sprague Dawley rats
Rat	Sprague-Dawley	Whole Brain Lysate	Unspecified	Rat Brain Whole Tissue Lysate (Post-Natal Whole)

4 Assay Performance and Reproducibility

Figure 1

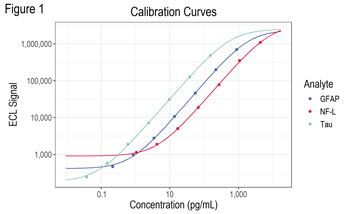


Figure 2

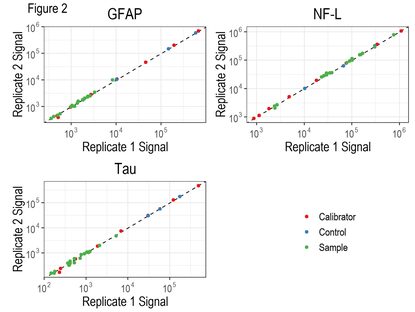


Figure 1: Representative calibration curves of GFAP, NF-L and Tau.

Figure 2: Reproducibility plots of mouse samples for GFAP, NF-L and Tau.

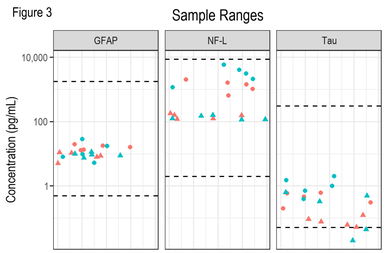
Table 2: Duplicate % CV range of mouse samples measured on the human neurology panel.

Table 2: Mouse Sample %CV

	Minimum	Q1	Median	Q3	Maximum
GFAP	0.02	0.81	2.0	3.6	19
NF-L	0.00	2.1	3.0	4.6	13
Tau	0.22	1.9	4.7	7.8	22

5 Rodent Native Analyte Recognition and Dilution Linearity

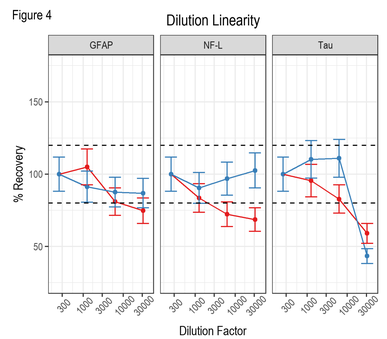
Figure 3



Normal BALB/C mouse and normal Sprague Dawley rat serum and plasma (n=5 each) samples were all detectable in the human GFAP and NF-L assays, and all but two samples were within the limits of quantitation in the human Tau assay (Figure 3).

Figure 3: Concentration ranges of normal mouse and rat serum and plasma samples. Dashed lines are dilution-adjusted (2-fold) quantitation limits.

Figure 4



Normal mouse and rat whole brain lysates were tested for dilution linearity and cross-reactivity. With an initial total protein concentration of 1mg/mL, the starting dilution factor was 250-fold with further serial dilutions to 31,250-fold. The recommended dilution is 250-fold to 1000-fold in whole brain lysate, which here is the equivalent of 1 – 4 µg/mL of total protein (Figure 4).

Normal mouse and rat serum and plasma were tested at 2-fold and 5-fold dilutions (data not shown). All analytes were detectable and diluted linearly in mouse serum and plasma and in rat serum. GFAP and NF-L were detectable and diluted linearly in rat plasma, while Tau was only detectable in rat plasma at 2-fold dilution. The recommended dilution for mouse and rat serum and plasma is 2-fold, aligning with the human serum and plasma recommendation for this assay.

Figure 4: Dilution linearity of whole brain lysate. Error bars are 95% CI. Dashed lines indicate ±20% from reference sample at 250-fold dilution.

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6 Elevated Neurology Biomarkers in Mouse Models of Neurodegeneration

Plasma from four different mouse models of Alzheimer's disease (AD) and one model of amyotrophic lateral sclerosis (ALS) were tested alongside C57BL/6 (control) commercially sourced mouse plasma at the recommended 2-fold dilution. Cerebrospinal fluid (CSF) samples matched from one model of AD and the model of ALS were tested at 100-fold dilution only due to limited sample availability. The biomarkers in all plasma samples were within the quantifiable range. The AD CSF samples were thresholded to assay limits of quantitation for GFAP and Tau, but detectable for NF-L. The ALS CSF samples showed elevated levels of all three analytes compared to levels in the AD model, though none showed significance due to high variability and the small number of samples that were tested. All plasma analytes trended higher in all ND models compared to normal mice, and measurements in the ALS model were further elevated above the AD model. AD plasma levels were significantly higher for GFAP and NF-L compared to controls.

Figure 5

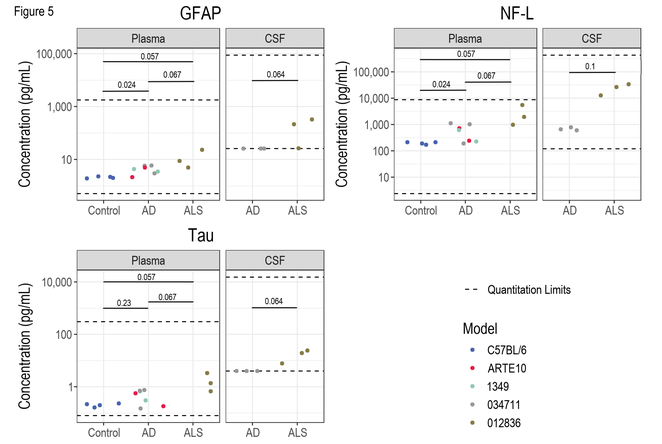

Figure 5: Plasma and CSF concentrations from control and ND mouse models. P-values calculated between differences of means by Wilcoxon test. Dashed lines indicate dilution-adjusted quantitation limits.

7 Matched Plasma and CSF Correlations in Mice

Levels of GFAP, NF-L and Tau in matched plasma and CSF from an AD mouse model (n=3) and an ALS mouse model (n=3) were highly correlated, indicating mouse plasma is a sufficient matrix for detecting biomarkers of neurodegeneration in these models. This is consistent with literature on matched human CSF and plasma levels of GFAP, NF-L and Tau.

Figure 6

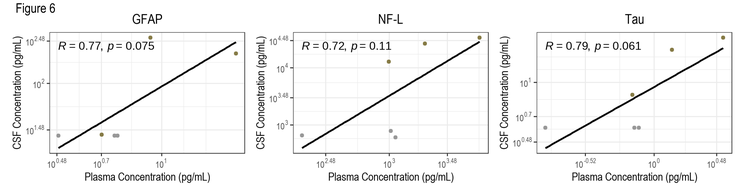

Figure 6: Matched plasma and CSF from ND mouse models correlated between 0.72-0.79 Pearson's coefficient across all three analytes.

8 Conclusions

The MSD Human S-PLEX Neurology Panel 1 effectively cross-reacts with mouse and rat samples. GFAP and NF-L are fully detectable in mouse and rat serum, plasma, brain lysate, and CSF, and tau is detectable at a 2-fold dilution for serum and plasma and in 0.16 µg/mL of total brain lysate protein. Neurodegenerative mouse models display measurable increases in all analytes, varying based on the particular model. The humanized TDP-43 mouse model of ALS expresses particularly high levels of GFAP, NF-L and Tau compared to all tested AD mouse models. Levels in matched plasma and CSF samples from ND mouse models correlate well for all three analytes. These data demonstrate that the MSD Human S-PLEX Neurology Panel 1 can be used to quantify GFAP, NF-L, and Tau in various rodent models for research, model development, and biomarker development studies.

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