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PUBLICATION SPOTLIGHT

LEARN HOW FULLY AUTOMATED WESTERNS ENABLE CUTTING-EDGE NEUROSCIENCE RESEARCH

WHY YOUR RESEARCH MATTERS TO US

Some of the most prevalent and least understood diseases are those that affect the nervous system. These range from neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, to psychiatric conditions, including depression and anxiety-related disorders. Advances in our understanding of the molecular mechanisms underlying nervous system dysfunction is critical for the development of effective treatments.

ProteinSimple offers a range of research tools to study the biological processes that accompany neurological/neurodegenerative disease. Simple Western™ assays on Jess™, Wes™, Peggy Sue™ or Sally Sue™ offer fully automated Western analysis by charge or size separation.

THE SIMPLE WESTERN ADVANTAGE

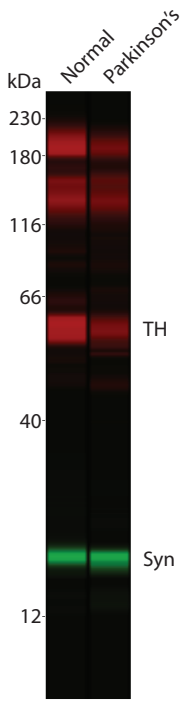
HIGH SENSITIVITY
- 3-5 µl sample
- Low pg detection

EASY TO USE
- Fully automated Westerns
- 3 hours to results

HIGH FLEXIBILITY
- Multiplex across chemiluminescence and fluorescence detection channels
- Analyze up to 96 samples at a time

Using Jess to confirm downregulation of tyrosine hydroxylase (TH) in Parkinson's disease.

With Jess, multiplexing another neuronal marker, in this case synuclein (Syn), is easy and provides a more meaningful picture of the biology.

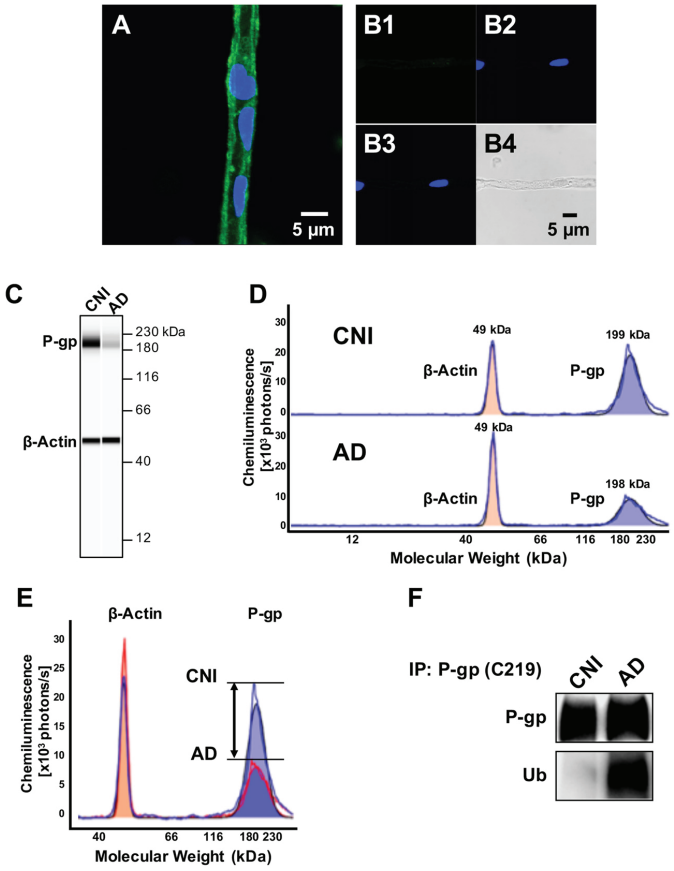


SIMPLE WESTERN ASSAYS IN THE HANDS OF YOUR PEERS

PREVENTING P-GP UBIQUITINATION LOWERS Aβ BRAIN LEVELS IN AN ALZHEIMER'S DISEASE MOUSE MODEL

P-glycoprotein (P-gp) is linked to Aβ clearance, and has important implications for Alzheimer's disease pharmacology, epidemiology and genetics. Here, Wes was used to measure P-gp expression.

P-gp expression levels are decreased and P-gp ubiquitination levels are increased in brain capillaries from Alzheimer's disease (AD) patients. (A) Representative image of a P-gp-immunostained (green) brain capillary isolated from brain tissue of a cognitive normal individual (CNI); nuclei were counterstained with DAPI (blue). (B) Negative control (no primary antibody): (B1; green channel); (B2; blue channel); (B3; overlay of green and blue channel) and (B4; transmitted light channel). (C) Representative Wes lane view image and (D) electropherograms showing reduced P-gp (blue shaded area) protein expression levels in brain capillaries isolated from human brain tissue (frontal cortex) of AD patients (n = 3) vs. CNIs (n = 3). (E) Overlay of electropherograms displayed in (D) show a reduction in the area under the curve (AUC) that represents P-gp protein expression levels (blue shaded area) in brain capillaries from AD patients (red line) relative to CNI (blue line). In contrast, β-actin levels (orange shaded area) in brain capillaries from AD patients (red line) and CNI (blue line) were the same. (F) Traditional Western blot showing that ubiquitin levels in P-gp-immunoprecipitates are increased in capillaries from AD patients compared to those from CNI. (Adapted from Hartz et al., 2018, CC BY 4.0)



A Hartz, Y Zhong, A Shen, E Abner, B Bauer, *Frontiers in Aging Neuroscience*, 2018; 10: 186.

Targets analyzed by Simple Western (SW): P-gp, β-actin. Sample type: normal and Alzheimer's disease brain homogenates.

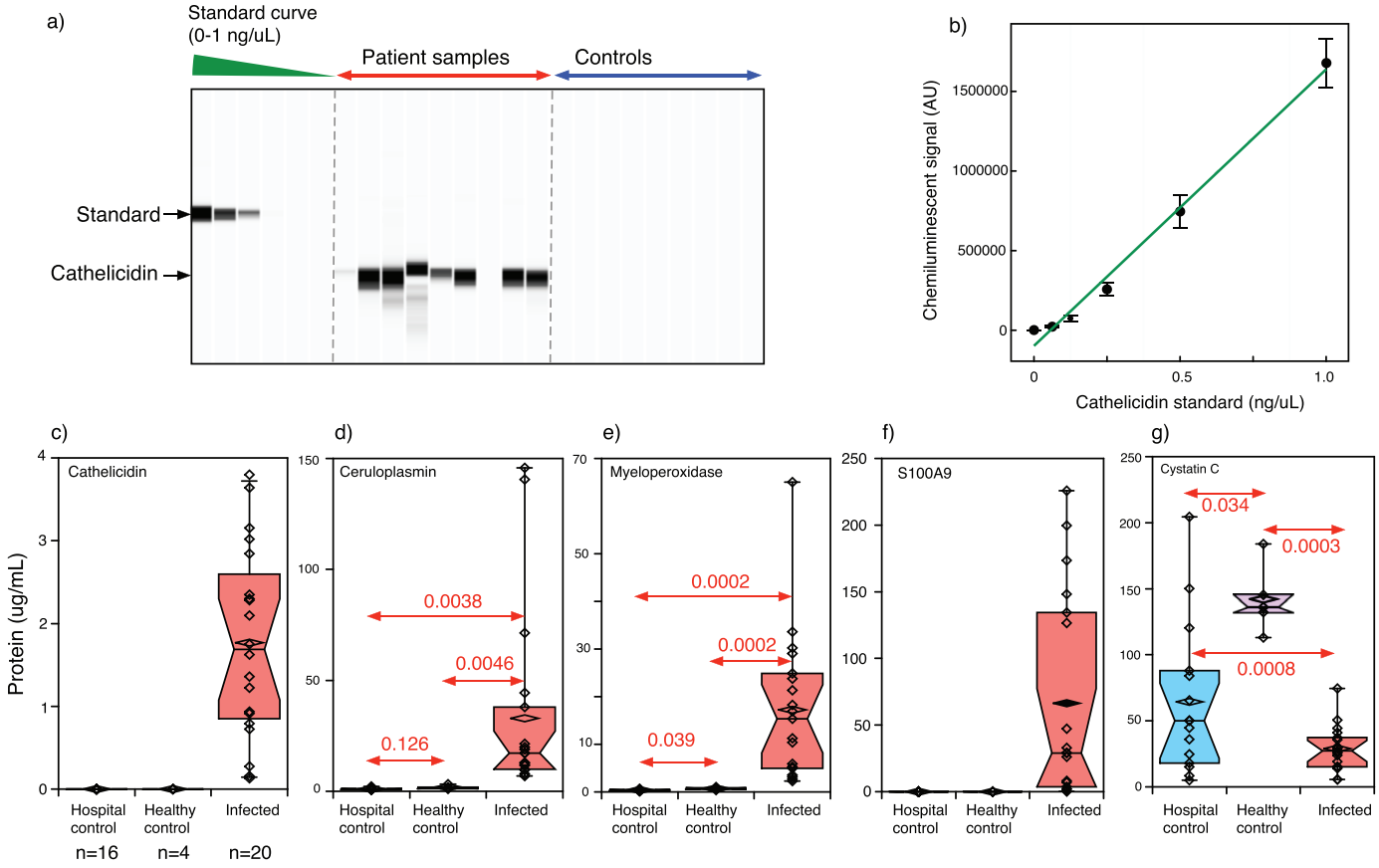
QUANTITATIVE PROTEOMICS OF CEREBROSPINAL FLUID IN PAEDIATRIC PNEUMOCOCCAL MENINGITIS

Proteomics data were confirmed with a Simple Western assay on Wes for 5 proteins that are elevated in cerebrospinal fluid (CSF) from patients with meningitis.

Automated Western blotting of selected CSF proteins. Five proteins, cathelicidin (panel c), ceruloplasmin (panel d), myeloperoxidase (panel e), protein S100A9 (panel f) and cystatin C (panel g), were quantified in a larger cohort (n = 40, 15 hospital controls, 5 healthy controls and 20 *S. pneumoniae* positive patients) using automated capillary Western blotting (Wes, ProteinSimple, CA, USA). A typical lane view image is shown in panel a, and the corresponding standard curve is shown in panel b. Purified recombinant proteins were used as calibration standards for all five proteins. Panels c to g are the summarised quantitative Western blot data for cathelicidin, ceruloplasmin, myeloperoxidase, S110A9 and cystatin C, respectively. (Adapted from Gómez-Baena et al., 2017, CC BY 4.0)

G Gómez-Baena, R Bennett, C Martínez-Rodríguez, M Wnęk, G Laing, G Hickey, L McLean, R Beynon, E Carrol, *Scientific Reports*, 2017; 7(1): 7042.

Targets analyzed by SW: cathelicidin, ceruloplasmin, myeloperoxidase, S110A9 and cystatin. Sample type: CSF fluid.



ADDITIONAL HIGHLIGHTED NEUROSCIENCE PUBLICATIONS USING SIMPLE WESTERN

1. In vivo gene editing in dystrophic mouse muscle and muscle stem cells, M Tabebordbar, K Zhu, J Cheng, W Chew, J Widrick, W Yan, C Maesner, E Wu, R Xiao, F Ran, L Cong, F Zhang, L Vandenberghe, G Church, A Wagers, *Science*, 2016; 351(6271): 407. **Targets analyzed by SW: dystrophin, vinculin. Sample type: AAV-Dmd CRISPR treated mouse muscle lysates.**

2. A divalent siRNA chemical scaffold for potent and sustained modulation of gene expression throughout the central nervous system, J Alterman, B Godinho, M Hassler, C Ferguson, D Echeverria, E Sapp, R Haraszti, A Coles, F Conroy, R Miller, L Roux, P Yan, E Knox, A Turanov, R King, G Gernoux, C Mueller, H Gray-Edwards, R Moser, N Bishop, S Jaber, M Gounis, M Sena-Esteves, A Pai, M DiFiglia, N Aronin, A Khvorova, *Nature Biotechnology*, 2019; 37(8): 884-894. **Targets analyzed by SW: APOE, vinculin. Sample type: mouse brain lysates.**

3. The effect of folic acid deficiency on FGF pathway via Brachyury regulation in neural tube defects, S Chang, X Lu, S Wang, Z Wang, J Huo, J Huang , S Shangguan, S Li, J Zou, Y Bao, J Guo, F Wang, B Niu, T Zhang, Z Qiu, J Wu, L Wang, *The FASEB Journal*, 2019; 33(4): 4688-4702. **Targets analyzed by SW: DUSP6, GRBS, MEK, ERK, β-actin, FGF8, FGFR, RAS, AKT. Sample type: mouse, human brain tissue homogenates.**

4. Neuron-specific genome modification in the adult rat brain using CRISPR-Cas9 transgenic rats, S Bäck, J Necarsulmer, L Whitaker, L Coke, P Koivula, E Heathward, L Fortuno, Y Zhang, C Yeh, H Baldwin, M Spencer, C Mejias-Aponte, J Pickel, A Hoffman, C Spivak, C Lupica, S Underhill, S Amara, A Domanskyi, J Anttila, M Airavaara, B Hope, F Hamra, C Richie, B Harvey, *Neuron*, 2019; 102(1): 105-119.e8. **Targets analyzed by SW: MANF, actin. Sample type: rat primary cortical neurons.**

5. Tau protein aggregation is associated with cellular senescence in the brain, N Musi, J Valentine, K Sickora, E Baeuerle, C Thompson, Q Shen, M Orr, *Aging Cell*, 2018; 17(6): e12840-e12840. **Targets analyzed by SW: tau, gH2AX, NFKb p65. Sample type: mouse forebrain homogenates, human cortical brain homogenates.**

6. Application of high-throughput, capillary-based Western analysis to modulated cleavage of the cellular prion protein, A Castle, N Daude, S Gilch, D Westaway, *The Journal of Biological Chemistry*, 2019; 294(8): 2642-2650. **Targets analyzed by SW: PrPc, β-tubulin. Sample type: S3-3 RK13 cell lysates.**

7. Reduced AMPK activation and increased HCAR activation drive anti-inflammatory response and neuroprotection in glaucoma, M Harun-Or-Rashid, D Inman, *Journal of Neuroinflammation*, 2018; 15(1) 313. **Targets analyzed by SW: Iba1. Sample type: mouse retina and optic nerve tissues.**

8. The attenuating effects of 1,2,3,4,6 penta-O-galloyl-β-d-glucose on pro-inflammatory responses of LPS/IFNy-activated BV-2 microglial cells through NFκB and MAPK signaling pathways, P Mendonca, E Taka, D Bauer, R Reams, K Soliman, *Journal of Neuroimmunology*, 2018; 324: 43-53. **Targets analyzed by SW: p-IRAK1, p-CHUK, p-NFKb1, p-CDK2, GAPDH. Sample type: BV-2 microglial cell lysates.**

9. Plasminogen activator inhibitor-1 reduces tissue-type plasminogen activator–dependent fibrinolysis and intrahepatic hemorrhage in experimental acetaminophen overdose, A Pant, A Kopec, K Baker, H Cline-Fedewa, D Lawrence, J Luyendyk, *The American Journal of Pathology*, 2018; 188(5): 1204-1212. **Targets analyzed by SW: APAP. Sample type: hepatic tissue from WT and PAI-1 -/- mice.**

10. Detection of CSF 14-3-3 protein in sporadic Creutzfeldt-Jakob disease patients using a new automated capillary Western assay, A Fourier, A Dorey, A Perret-Liaudet, I Quadrio, *Molecular Neurobiology*, 2018; 55(4): 3537-3545. **Targets analyzed by SW: 14-3-3. Sample type: cerebrospinal fluid.**

11. Glutamate-glutamine transfer and chronic stress-induced sex differences in cocaine responses, A Shimamoto, V Rappeneau, H Munjal, T Farris, C Davis, A Wilson, M Edwards, C Moore, C Reynolds, C Meshul, *Neuroscience*, 2018; 391: 104-119. **Targets analyzed by SW: SNAT1/2, PAG, GLT-1, NSE, GAPDH, Na+/K+ ATPase. Sample type: rat brain lysates (plasma membrane and cytosol fractions).**

12. Proteomic analysis of aged microglia: shifts in transcription, bioenergetics, and nutrient response, A Flowers, H Bell-Temin, A Jalloh, S Stevens, P Bickford, *Journal of Neuroinflammation*, 2017; 14(1): 96. **Targets analyzed by SW: RICTOR, pAKT, AKT, GAPDH. Sample type: primary microglia.**

13. Vitronectin from brain pericytes promotes adult forebrain neurogenesis by stimulating CNTF, C Jia, M Keasey, H Malone, C Lovins, R Sante, V Razskazovskiy, T Hagg, *Experimental Neurology*, 2019; 312: 20-32. **Targets analyzed by SW: pFAK, a-tubulin. Sample type: mouse brain homogenates.**

14. The proneural gene ASCL1 governs the transcriptional subgroup affiliation in glioblastoma stem cells by directly repressing the mesenchymal gene NDRG1, A Narayanan, F Gagliardi, A Gallotti, S Mazzoleni, M Cominelli, L Fagnocchi, M Pala, I Piras, P Zordan, N Moretta, E Tratta, G Brugnara, L Altabella, G Bozzuto, P Gorombei, A Molinari, R Padua, A Bulfone, L Politi, A Falini, A Castellano, P Mortini, A Zippo, P Poliani, R Galli, *Cell Death & Differentiation*, 2019; 26(9): 1813-1831. **Targets analyzed by SW: NDRG1, pNDRG1. Sample type: cancer stem cell lines L0605, L0512.**

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