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a bio-techne brand

PUBLICATION SPOTLIGHT

SIMPLE WESTERN CHARGE ASSAYS ARE ENABLING CUTTING EDGE BIOMEDICAL RESEARCH

WHY YOUR RESEARCH MATTERS TO US

Characterizing the charge heterogeneity of biomolecules can provide profound insight into biological processes. For example, a protein's charge heterogeneity will change in response to post-translational modifications like phosphorylation, and these changes may not be readily detectable by size-based assays like the traditional Western blot. Simple Western™ Charge assays separate proteins by capillary isoelectric focusing based on their pI and can be seamlessly followed up with a highly sensitive immunoassay detection and analysis. Simple Western Charge assays have been used extensively in biomedical research, creating charge heterogeneity fingerprints used to study topics related to cancer, neurological disease, diabetes and more.

Simple Western Charge assays on Peggy Sue™ or NanoPro 1000™ analyze up to 96 native, non-denatured samples in a fully automated fashion, and the data generated are reproducible and quantitative. Simple Western Charge assays use as little as 0.2 µg/µL of protein in just 5 µL of sample, and those few microliters can be interrogated up to eight times! This means you'll get a large amount of data from a very small sample size.

THE SIMPLE WESTERN ADVANTAGE

TIME TO RESULT
- Fully automated charge-based immunoassays
- Comprehensive analysis and results overnight

QUANTITATION
- Built in analysis software
- Absolute and relative protein quantitation

THROUGHPUT AND FLEXIBILITY
- Up to 96 samples per run
- Wide gradient ranges from pI 3 to pI 10
- Compatible with primary tissue or fine-needle aspirate samples

REPRODUCIBILITY
- Low inter- and intra-assay CVs

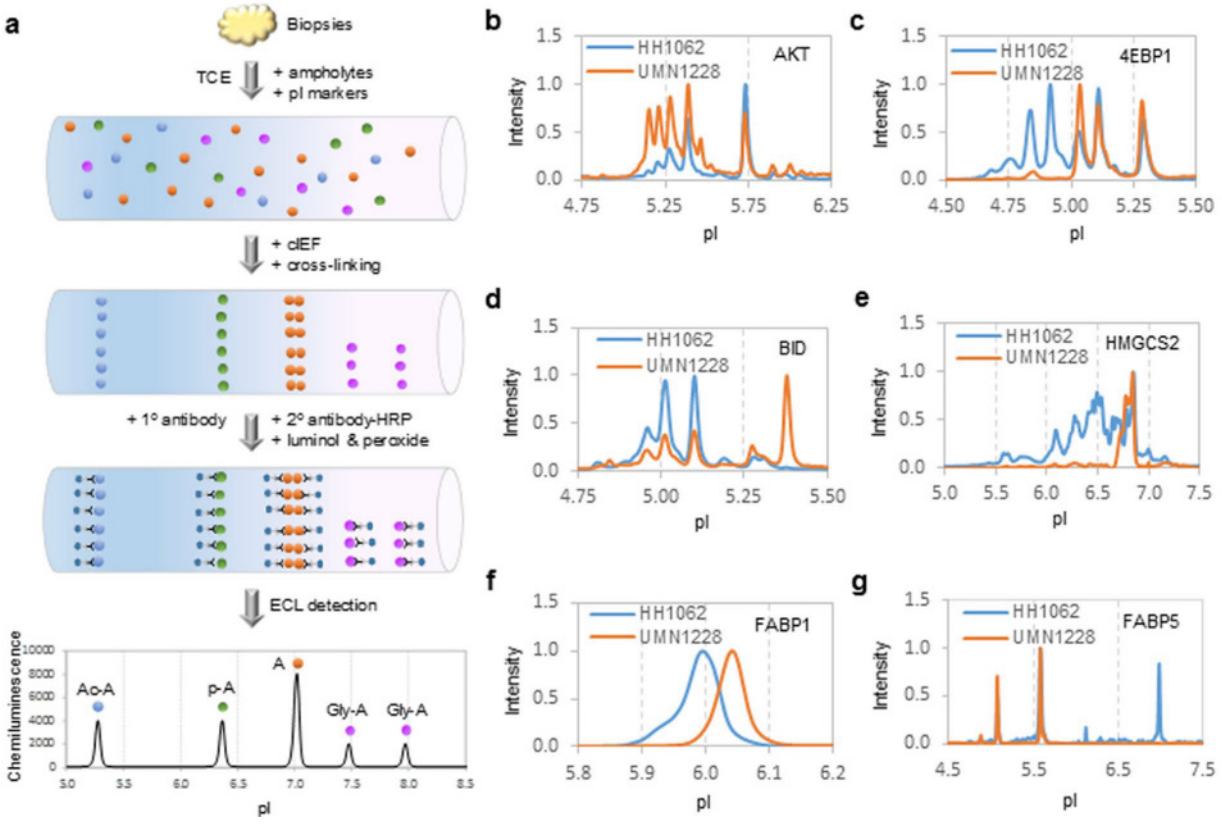
LOW SAMPLE VOLUME
- Start from as little as 0.6 µg or 6 µl per well to get pg-level sensitivity

SIMPLE WESTERN CHARGE ASSAYS IN THE HANDS OF YOUR PEERS

QUANTITATIVE ASSESSMENT OF LIVER STEATOSIS AND AFFECTED PATHWAYS WITH MOLECULAR IMAGING AND PROTEOMIC PROFILING

Current assessment of non-alcoholic fatty liver disease (NAFLD) with histology is time-consuming, insensitive to early-stage detection, qualitative, and lacks information on etiology. This study showed that NanoPro 1000 quantitatively measured perturbations to the post-translational modification profiles of selective liver proteins to identify affected cellular signaling and metabolic pathways in a few hours. (Adapted from Urasaki et al., 2018, CC BY 4.0)

Y Urasaki, C Zhang, J Cheng and T Le, *Scientific Reports*, 2018; 8(1):3606



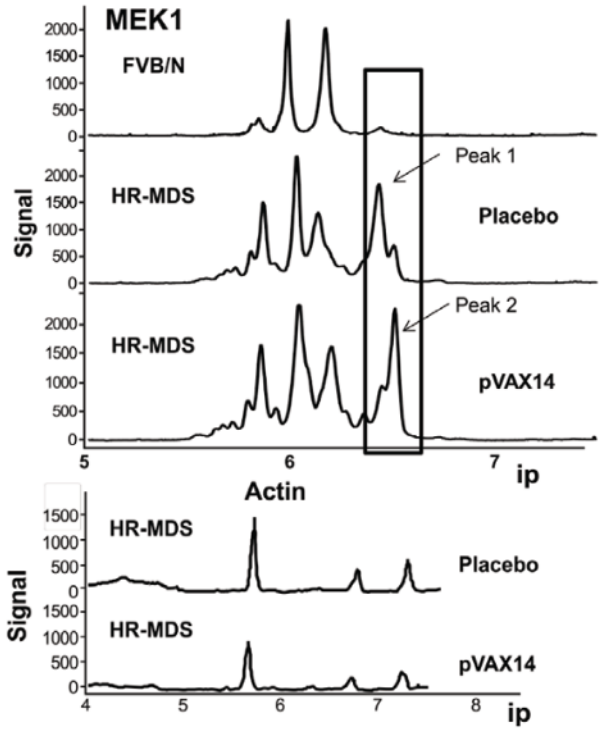
Profiling of selective liver protein species with NanoPro 1000. (a) Experimental flow of a typical IEF immunoassay to detect protein A species in a single capillary. TCE: total cell extract; pI: isoelectric point; cIEF: capillary isoelectric focusing; HRP: horseradish peroxidase; ECL: enhanced chemiluminescence; Ac-A: acetyl-isoforms; p-A: phosphor-isoforms; Gly-A: glycosyl-isoforms. (b–g) Representative cIEF electropherograms of (b) Akt, (c) 4EBP1, (d) BID, (e) HMGCS2, (f) FABP1, and (g) FABP5 in a control liver (HH1062, blue lines) versus a non-alcoholic steatohepatitis liver (UMN1228, orange lines).

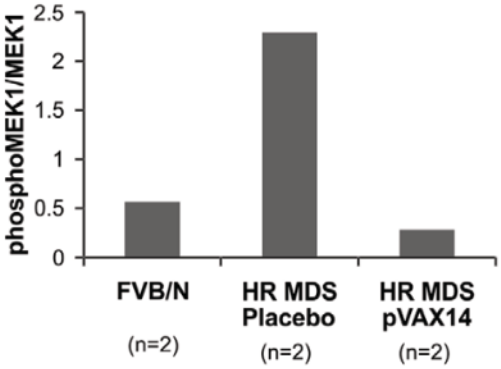
DNA-MEDIATED ADJUVANT IMMUNOTHERAPY EXTENDS SURVIVAL IN TWO DIFFERENT MOUSE MODELS OF MYELOID MALIGNANCIES

This study reports the efficacy of a non-specific DNA vaccine, pVAX14, in both acute promyelocytic leukemia and high-risk myelodysplastic syndrome (HR-MDS) models. In HR-MDS mice, pVAX14 significantly reduced biomarkers of leukemic transformation such as phosphorylated mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) 1. These MEK1 phosphorylated isoforms were clearly resolved on NanoPro 1000. (Adapted from Le Pogam et al., 2015, CC BY 3.0)

C Le Pogam, S Patel, P Gorombei, L Guerenne, P Krief, N Omidvar, N Tekin, E Bernasconi, F Sicre, M Schlageter, M Chopin, M Noguera, R West, A Abu, V Mathews, M Pla, P Fenaux, C Chomienne and R Padua, *Oncotarget*, 2015; 6(32):32494–32508.

Representative NanoPro 1000 traces showing MEK1 spleen extracts from normal (FVB/N) and HR-MDS treated with either placebo (HBSS) or pVAX14. The isoelectric points (ip) are shown on the x-axis. Boxed are peak 1 representing a phosphorylated isoform (*arrowed*) and peak 2, a dephosphorylated isoform (*arrowed*). Quantitative histograms (n=2 in triplicate) are expressed as a ratio of pMEK1/MEK1. Actin was used as a housekeeping protein to control for protein loading of samples derived from mice treated with placebo and or with pVAX14.





ADDITIONAL PUBLICATION SPOTLIGHTS FOR SIMPLE WESTERN CHARGE ASSAYS

1. Nano-fluidic proteomic assay for serial analysis of oncoprotein activation in clinical specimens, A Fan, D Deb-Basu, M Orban, J Gotlib, Y Natkunam, R O'Neill, R Padua, L Xu, D Taketa, A Shirer, S Beer, A Yee, D Voehringerand D Felsher, *Nature Medicine,* 2009; 15(5): 566–571.**Sample type:** human tumor cells including Burkitt lymphomas, T-cell acute lymphoblastic leukemia, follicular lymphomas, chronic myelogeneous leukemia and chronic lymphocytic leukemia **Targets:** MYC, BCL2, activated caspase 3, ERK1/2, pERK1/2, MEK1/2, pMEK1, pSTAT3/5, pJNK, HSP70

2. 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 and R Galli, *Cell Death and Differentiation,* 2019; 26(9):1813–1831. **Sample type:** glioblastoma cancer stem cells **Targets:** NDRG1, pNDRG1

3. Loss of Lkb1 impairs Treg function and stability to aggravate graft-versus-host disease after bone marrow transplantation, X Su, Q Wang, W Guo, X Pei, Q Niu, M Liu, Y Liu, S Chen, S Feng, Y He, D Yang, R Zhang, Q Ma, W Zhai, A Pang, J Wei, Y Huang, Y Luo, M Han, X Feng and E Jiang, *Cellular & Molecular Immunology,* 2019. **Sample type:** human regulatory T cells **Targets:** Lkb1, GAPDH

4. Tcf7l1 acts as a suppressor for the self-renewal of liver cancer stem cells and is regulated by IGF/MEK/ERK signaling independent of ß-Catenin, J Shan, J Shen, M Wu, H Zhou, J Feng, C Yao, Z Yang, Q Ma, Y Luo, Y Wang and C Qian, *Stem Cells,* 2019; 37(11):1389–1400. **Sample type:**human hepatocellular carcinoma cell lines Huh7 and PLC/PRF/5 **Target:** Tcf7l1

5. Facilitation of MrgprD by TRP-A1 promotes neuropathic pain, C Wang, L Gu, Y Ruan, X Geng, M Xu, N Yang, L Yu, Y Jiang, C Zhu, Y Yang, Y Zhou, X Guan, W Luo, Q Liu, X Dong, G Yu, L Lan and Z Tang, *FASEB Journal,* 2019; 33(1):1360–1373. **Sample type:** mouse cultured dorsal root ganglia neurons**Target:** TRP-A1

6. CD106 is a novel mediator of bone marrow mesenchymal stem cells via NF-κB in the bone marrow failure of acquired aplastic anemia, S Lu, M Ge, Y Zheng, J Li, X Feng, S Feng, J Huang, Y Feng, D Yang, J Shi, F Chen and Z Han, *Stem Cell Research and Therapy,* 2017; 8:178. **Sample type:** human bone marrow mesenchymal stem cells **Target:** NF-κB (p65)

7. Molecular targeting of the Aurora-A/SMAD5 oncogenic axis restores chemosensitivity in human breast cancer cells, M Opyrchal, M Gil, J Salisbury, M Goetz, V Suman, A Degnim, J McCubrey, T Haddad, I Iankov, C Kurokawa, N Shumacher, J Ingle, E Galanis and A D'Assoro, *Oncotarget,* 2017; 8(53):91803–91816. **Sample type:** human breast cancer cell line MDA-MB-231 **Target:** p~Aurora-A, p~SMAD5

8. Mitigation of reversible self-association and viscosity in a human IgG1 monoclonal antibody by rational, structure-guided Fv engineering, J Geoghegan, R Fleming, M Damschroder, S Bishop, H Sathish and R Esfandiary,*mAbs,* 2016; 8(5):941–950. **Sample type:** human IgG1 monoclonal antibody **Target:** human IgG Fc

9. Inositol-C2-PAF acts as a biological response modifier and antagonizes cancer-relevant processes in mammary carcinoma cells, C Pelz, S Häckel, G Semini, S Schrötter, W Bintig, S Stricker, G Mrawietz, A Klein, L Lucka, V Shmanai, B Eickholt, A Hildmann and K Danker, *Cellular Oncology,* 2018; 41(5):505–516. **Sample type:** human breast cancer cell line MCF-7 **Targets:** pAkt (S473)

10. A specific PTPRC/CD45 phosphorylation event governed by stem cell chemokine CXCL12 regulates primitive hematopoietic cell motility,A Williamson, A Pierce, E Jaworska, C Zhou, M Aspinall-O'Dea, L Lancashire, R Unwin, S Abraham, M Walker, S Cadecco, E Spooncer, T Holyoake and A Whetton, *Molecular and Cellular Proteomics,* 2013; 12(11):3319–3329. **Sample type:** clinical CD34+ cells **Target:** pS962 PTPRC/CD45

11. Saliva as a medium to detect and measure biomarkers related to pain, H Jasim, A Carlsson, B Hedenberg-Magnusson, B Ghafouri and M Ernberg, *Scientific Reports,* 2018; 8:3220. **Sample type:**saliva and plasma **Targets:** BDNF, NGF and CGRP

12. Modified serpinA1 as risk marker for Parkinson's disease dementia: Analysis of baseline data,S Halbgebauer, M Nagl, H Klafki, U Haußmann, P Steinacker, P Oeckl, J Kassubek, E Pinkhardt, A Ludolph, H Soininen, S Herukka, J Wiltfang and M Otto, *Scientific Reports,* 2016; 6:26145. **Sample type:** cerebrospinal fluid **Target:** SerpinA1

13. Highly sensitive and specific protein detection via combined capillary isoelectric focusing and proximity ligation,N Padhan, J Yan, A Boge, E Scrivener, H Birgisson, A Zieba, M Gullberg, M Kamali-Moghaddam, L Claesson-Welsh and U Landegren, *Scientific Reports,* 2017; 7:1490. **Sample type:** human colorectal cancer cells and dermal microvascular endothelial cells **Targets:** pERK1/2, pMEK1, pSrc Y418, aldose reductase, S100 A6, CDC34

14. High sensitivity isoelectric focusing to establish a signaling biomarker for the diagnosis of human colorectal cancer, N Padhan, T Nordling, M Sundström, P Åkerud, H Birgisson, P Nygren, S Nelander and L Claesson-Welsh, *BMC Cancer,* 2016; 16:683. **Sample type:** human colorectal cancer cells **Target:** ERK1/2, pERK1/2, PLCy1, AKT, p70S6 kinase, MEK 1/2, c-SRC, EGFR, HSP 70

15. Pancreatic-ß-cell survival and proliferation are promoted by protein kinase G type Ia and downstream regulation of AKT/FOXO1, J Wong, V Vo, P Gorjala and R Fiscus, *Diabetes and Vascular Disease Research,* 2017; 14(5):434–449. **Sample type:** primary cultures of pancreatic islets from mice, two pancreatic ß-cell lines (RINm5F and Beta-TC-6) and pancreatic ductal carcinoma PANC-1 **Target:** PKG-Ia and PKG-Iß isoforms

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