

# Odyssey® Publications

## In-Cell Western™ Assays

1/2007 – 6/2009

Summer 2009

Volume 1

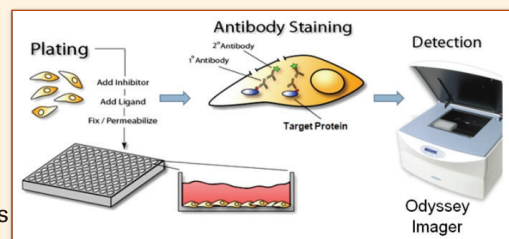
Selected From Over 70 publications

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### In-Cell Western

The In-Cell Western (ICW) assay is similar to standard immunocytochemical methods. The assay can be performed in 96- or 384-well plates. The cells are fixed and stained with, for example, phospho- and pan-antibodies from different species followed by species-specific secondary antibodies labeled with different IRDye® infrared dyes. Alternatively, the cells can be stained with a target-specific antibody and a nuclear stain like DRAQ V. The use of two colors available on Odyssey allows ratiometric normalization across the wells of the plate and yields a quantitative assessment of the level of target protein. The quantification accuracy is also maximized by normalization to account for differences in cell number from well to well. As there is very little autofluorescence from either cellular materials or plastics in the NIR region, the signal-to-noise ratio is relatively high. Multiple markers, pathways or drug compounds can be examined on each plate.



The ICW assay has been used for:

1. Protein trafficking for cell surface proteins including ion channels (Chang *et al.* 2008; Zhang *et al.* 2007) hormone receptors (Sharifi *et al.* 2008), and tissue factors (Egorina *et al.* 2006)
2. Analysis of protein signaling pathways, including the effects of drugs on multiple points within one or more signaling pathways (Kumar *et al.* 2008).
3. Cell-based determinations of IC<sub>50</sub> concentrations in the secondary screening of lead compounds (Hannoush 2008; Selkirk *et al.* 2006).
4. RNAi studies (Arrendondo *et al.* 2008)
5. Cell proliferation assay (Montagut *et al.* 2008)

### Activation of Src by Protein Tyrosine Phosphatase 1B Is Required for ErbB2 Transformation of Human Breast Epithelial Cells

Luis E. Arias-Romero, Sayanti Saha, Olga Villamar-Cruz, Shu-Chin Yip, Stephen P. Ethier, Zhong-Yin Zhang, and Jonathan Chernoff  
*Cancer Res.*, Jun 2009; 69: 4582 - 4588.

### Role of activated endocannabinoid system in regulation of cellular cholesterol metabolism in macrophages

Li-sheng Jiang, Jun Pu, Zhi-hua Han, Liu-hua Hu, and Ben He  
*Cardiovasc Res.*, Mar 2009; 81: 805 - 813.

### Fibroblast growth factor receptor 1 is a key regulator of early adipogenic events in human preadipocytes

C. H. Widberg, F. S. Newell, A. W. Bachmann, S. N. Ramnøruth, M. C. Spelta, J. P. Whitehead, L. J. Hutley, and J. B. Prins  
*Am J Physiol Endocrinol Metab.*, Jan 2009; 296: E121 - E131.

## **In-Cell Western (Continued)**

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### **Disease-causing Mutation in GPR54 Reveals the Importance of the Second Intracellular Loop for Class A G-protein-coupled Receptor Function**

Jennifer L. Wacker, David B. Feller, Xiao-Bo Tang, Mia C. DeFino, Yuree Namkung, John S. Lyssand, Andrew J. Mhyre, Xu Tan, Jill B. Jensen, and Chris Hague  
*J. Biol. Chem.*, Nov 2008; 283: 31068 - 31078.

### **Carbon Monoxide and Nitric Oxide Mediate Cytoskeletal Reorganization in Microvascular Cells via Vasodilator-Stimulated Phosphoprotein Phosphorylation: Evidence for Blunted Responsiveness in Diabetes**

Sergio Li Calzi, Daniel L. Purich, Kyung Hee Chang, Aqeela Afzal, Takahiko Nakagawa, Julia V. Busik, Anupam Agarwal, Mark S. Segal, and Maria B. Grant  
*Diabetes*, Sep 2008; 57: 2488 - 2494.

### **Deoxynivalenol Induces p38 Interaction with the Ribosome in Monocytes and Macrophages**

Hee Kyong Bae and James J. Pestka  
*Toxicol. Sci.*, Sep 2008; 105: 59 - 66.

### **Airway smooth muscle cell tone amplifies contractile function in the presence of chronic cyclic strain**

Nigel J. Fairbank, Sarah C. Connolly, James D. MacKinnon, Kathrin Wehry, Linhong Deng, and Geoffrey N. Maksym  
*Am J Physiol Lung Cell Mol Physiol*, Sep 2008; 295: L479 - L488.

### **N-Palmitoyl Glycine, a Novel Endogenous Lipid That Acts As a Modulator of Calcium Influx and Nitric Oxide Production in Sensory Neurons**

Neta Rimmerman, Heather B. Bradshaw, H. Velocity Hughes, Jay Shih-Chieh Chen, Sherry Shu-Jung Hu, Douglas McHugh, Eivind Vefring, Jan A. Jahnsen, Eric L. Thompson, Kim Masuda, Benjamin F. Cravatt, Sumner Burstein, Michael R. Vasko, Anne L. Prieto, David K. O'Dell, and J. Michael Walker  
*Mol. Pharmacol.*, Jul 2008; 74: 213 - 224.

### **Identification and characterization of NVP-BE2235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent *in vivo* antitumor activity**

Sauveur-Michel Maira, Frédéric Stauffer, Josef Brueggen, Pascal Furet, Christian Schnell, Christine Fritsch, Saskia Brachmann, Patrick Chène, Alain De Pover, Kevin Schoemaker, Doriano Fabbro, Daniela Gabriel, Marjo Simonen, Leon Murphy, Peter Finan, William Sellers, and Carlos Garcia-Echeverria  
*Mol. Cancer Ther.*, Jul 2008; 7: 1851 - 1863.

### **Vaccinia Virus DNA Ligase Recruits Cellular Topoisomerase II to Sites of Viral Replication and Assembly**

Y.-C. James Lin, Jianhong Li, Chad R. Irwin, Heather Jenkins, Luke DeLange, and David H. Evans  
*J. Virol.*, Jun 2008; 82: 5922 - 5932.

### **Receptor-mediated tobacco toxicity: acceleration of sequential expression of $\alpha 5$ and $\alpha 7$ nicotinic receptor subunits in oral keratinocytes exposed to cigarette smoke**

Juan Arredondo, Alexander I. Chernyavsky, David L. Jolkovsky, Kent E. Pinkerton, and Sergei A. Grando  
*FASEB J*, May 2008; 22: 1356 - 1368.

### **Lipoprotein Receptor-Related Protein-1 Mediates Amyloid- $\beta$ -Mediated Cell Death of Cerebrovascular Cells**

Micha M.M. Wilhelmus, Irene Otte-Höller, Jos J.J. van Triel, Robert Veerhuis, Marion L.C. Maat-Schieman, Guojun Bu, Robert M.W. de Waal, and Marcel M. Verbeek  
*Am. J. Pathol.*, Dec 2007; 171: 1989 - 1999.

### **Proteinase-Activated Receptor-2 Exerts Protective and Pathogenic Cell Type-Specific Effects in Alzheimer's Disease**

Amir Afkhami-Goli, Farshid Noorbakhsh, Avril J. Keller, Nathalie Vergnolle, David Westaway, Jack H. Jhamandas, Patricia Andrade-Gordon, Morley D. Hollenberg, Hossein Arab, Richard H. Dyck, and Christopher Power  
*J. Immunol.*, Oct 2007; 179: 5493 - 5503.

### **MAGE-A, mMage-b, and MAGE-C Proteins Form Complexes with KAP1 and Suppress p53-Dependent Apoptosis in MAGE-Positive Cell Lines**

Bing Yang, Sean M. O'Herrin, Jianqiang Wu, Shannon Reagan-Shaw, Yongsheng Ma, Kumar M.R. Bhat, Claudia Gravekamp, Vijayasradhi Setaluri, Noel Peters, F. Michael Hoffmann, Hongzhuang Peng, Alexey V. Ivanov, Andrew J.G. Simpson, and B. Jack Longley  
*Cancer Res.*, Oct 2007; 67: 9954 - 9962.

### **Cyclosporin A increases expression of matrix metalloproteinase 9 and 2 and invasiveness *in vitro* of the first-trimester human trophoblast cells via the mitogen-activated protein kinase pathway**

Wen-Hui Zhou, Mei-Rong Du, Lin Dong, Xiao-Yong Zhu, Jin-Ying Yang, Ying-Yan He, and Da-Jin Li  
*Hum. Reprod.*, Oct 2007; 22: 2743 - 2750.

## In-Cell Western (Continued)

### Reactive oxygen species and the regulation of renal Na<sup>+</sup>-K<sup>+</sup>-ATPase in opossum kidney cells

Elisabete Silva and Patrício Soares-da-Silva

*Am J Physiol Regulatory Integrative Comp Physiol*, Oct 2007; 293: R1764 - R1770.

### Differentially Alters CNS Responses to IL-1 Depending on Its Route of Administration

San Ching, Hao Zhang, Natalya Belevych, Lingli He, Wenmin Lai, Xin-an Pu, Laura B. Jaeger, Qun Chen, and Ning Quan  
*J. Neurosci.*, Sep 2007; 27: 10476 - 10486.

### The ATM/ATR Signaling Effector Chk2 Is Targeted by Epstein-Barr Virus Nuclear Antigen 3C To Release the G2/M Cell Cycle Block

Tathagata Choudhuri, Subhash C. Verma, Ke Lan, Masanao Murakami, and Erle S. Robertson

*J. Virol.*, Jun 2007; 81: 6718 - 6730.

### Solenopsin, the alkaloidal component of the fire ant (*Solenopsis invicta*), is a naturally occurring inhibitor of phosphatidylinositol-3-kinase signaling and angiogenesis

Jack L. Arbiser, Tweeny Kau, Martha Konar, Krishna Narra, Ramani Ramchandran, Scott A. Summers, Chris J. Vlahos, Keqiang Ye, Betsy N. Perry, William Matter, Anthony Fischl, James Cook, Pamela A. Silver, Jenny Bain, Philip Cohen, David Whitmire, Scott Furness, Baskaran Govindarajan, and J. Phillip Bowen

*Blood*, Jan 2007; 109:560 - 565

## On-Cell Western

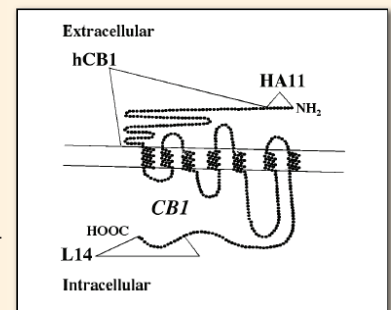
### On-Cell Western for cell surface protein trafficking

The On-Cell Western (OCW) assay allows the quantification of a protein on the surface of a cell. This was accomplished by comparing the signal from cells that were permeabilized with those that were not. It is also advantageous to use two different assays, one targeting an extracellular epitope and one targeting an intracellular epitope for a membrane protein.

Miller developed the OCW assay to study the internalization and recycling of the G-coupled protein receptor, CB1 (cannabinoid receptor). Being able to quantify the amounts of protein on the surface and inside a cell is essential to understanding cellular sensitivity to extracellular ligands. (See Daigle, *et al.* below, and LI-COR Application Note.)

The OCW has been employed to study a variety of receptors.

- Chan *et al.* used the OCW or "fluorescent cell surface assay" to study the trafficking of various mutant ACTH receptors to the cell surface.
- Chang *et al.* referred to the OCW as a "cell surface ELISA" and used it to look at the ability of drugs to rescue the cell surface expression of mutant CFTR protein.
- Egorina *et al.* referred to the assay as "Quantitative Immunofluorescent Staining" and used it to characterize total and surface tissue factor on mononuclear cells in response to LPS.
- Gonzalez-Gronow *et al.* employed the OCW to quantify knockdown of glucose regulated protein 78 (GRP78) expression on the cell surface following transfection of specific siRNAs.
- Markovic *et al.* used the assay to quantify the loss of Clathrin heavy chain (CRH-R2) receptors from the cell surface after agonist stimulation.



### Rapid CB1 cannabinoid receptor desensitization defines the time course of ERK1/2 MAP kinase signaling

Tanya L. Daigle, Christopher S. Kearns, and Ken Mackie

*Neuropharmacology* 54: 36 (2008)

### Functional consequence of a novel Y129C mutation in a patient with two contradictory melanocortin-2-receptor mutations

Li F. Chan, Teng-Teng Chung, Ahmed F. Massoud, Louise A. Metherell and Adrian J. L. Clark

*European Journal of Endocrinology* 160: 705 (2009)

### Role of N-linked oligosaccharides in the biosynthetic processing of the cystic fibrosis membrane conductance regulator

Xiu-bao Chang, April Mengos, Yue-xian Hou<sup>1</sup>, Liying Cui, Timothy J. Jensen, Andrei Aleksandrov John R. Riorda and Martina Gentsch

*J Cell Science* 121: 2814 (2008)

### In-Cell Western assay: a new approach to visualize tissue factor in human monocytes

E. M. Egorina, M. A. Sovershaev and B. Østerud

*Journal of Thrombosis and Haemostasis*, 4: 614-620

## On-Cell Western (Continued)

### Prostate Cancer Cell Proliferation *In vitro* Is Modulated by Antibodies against Glucose-Regulated Protein 78 Isolated from Patient Serum

Mario Gonzalez-Gronow, Miguel Cuchacovich, Carolina Llanos, Cristian Urzua, Govind Gawdi, and Salvatore V. Pizzo  
*Cancer Res*: 66: 23 (2006)

### Intracellular Mechanisms Regulating Corticotropin-Releasing Hormone Receptor-2\_ Endocytosis and Interaction with Extracellularly Regulated Kinase 1/2 and p38 Mitogen-Activated Protein Kinase Signaling Cascades

Danijela Markovic, Anu Punni, Hendrik Lehnert, and Dimitris K. Grammatopoulos  
*Molecular Endocrinology* 22:689–706 (2008)

### Direct interaction with filamins modulates the stability and plasma membrane expression of CFTR

William R. Thelin, Yun Chen, Martina Gentzsch, Silvia M. Kreda, Jennifer L. Sallee, Cameron O. Scarlett, Christoph H. Borchers, Ken Jacobson, M. Jackson Stutts, and Sharon L. Milgram  
*The Journal of Clinical Investigation* 117: 364 (2007)

### Distinct Melanocortin 2 Receptor Accessory Protein Domains Are Required for Melanocortin 2 Receptor Interaction and Promotion of Receptor Trafficking

Tom R. Webb, Li Chan, Sadani N. Cooray, Michael E. Cheetham, J. Paul Chapple, and Adrian J. L. Clark  
*Endocrinology* 150: 720 (2009)

## Live Cell Western

Delisle *et al.* have modified the OCW assay to quantitatively measure plasmalemmal expression of hERG in live cells. The data was consistent with Western blot and whole cell patch clamp analysis.

### Small gtpase determinants for the golgi processing and plasmalemmal expression of human ether-a-go-go related (hERG) K<sup>+</sup> channels

Brian P. Delisle, Heather A.S. Underkofler, Brooke M. Moungey, Jessica K. Slind, Jennifer A. Kilby, Jabe M. Best, Jason D. Foell, Ravi C. Balijepalli, Timothy J. Kamp, and Craig T. January  
*J. Biol. Chem.* 284: 2844 (2009)

## Cell Proliferation

The ICW has been adapted for cell proliferation or survival assays. A DNA stain such as SYTO<sup>®</sup> 60 is detected in the 700 nm channel to determine cell number. Widberg *et al.* validated the SYTO 60 assay against direct cell counts, and showed a strong positive correlation with cell number (R<sup>2</sup> = 0.9948).

### Fibroblast growth factor receptor 1 is a key regulator of early adipogenic events in human preadipocytes

C. H. Widberg, F. S. Newell, A. W. Bachmann, S. N. Ramnoruth, M. C. Spelta, J. P. Whitehead, L. J. Hutley, and J. B. Prins  
*Am J Physiol Endocrinol Metab* 296: E121–E131, 2009.

### Elevated CRAF as a Potential Mechanism of Acquired Resistance to BRAF Inhibition in Melanoma

Clara Montagut, Sreenath V. Sharma, Toshi Shioda, Ultan McDermott, Matthew Ulman, Lindsey E. Ulkus, Dora Dias-Santagata, Hannah Stubbs, Diana Y. Lee, Anurag Singh, Lisa Drew, Daniel A. Haber, and Jeffrey Settleman  
*Cancer Res* 68: 4853 (2008)

### The T790M “gatekeeper” mutation in EGFR mediates resistance to low concentrations of an irreversible EGFR inhibitor

Nadia Godin-Heymann, Lindsey Ulkus, Brian W. Brannigan, Ultan McDermott, Jennifer Lamb, Shyamala Maheswaran, Jeffrey Settleman, and Daniel A. Haber  
*Mol Cancer Ther.* 7: 874 (2008)

### Activated Kras, but Not Hras or Nras, May Initiate Tumors of Endodermal Origin via Stem Cell Expansion

Margaret P. Quinlan, Steven E. Quatela, Mark R. Philips, and Jeffrey Settleman  
*Molecular and Cellular Biology* 28: 2659 (2008)

### Mutations in the neutral sphingomyelinase gene SMPD3 implicate the ceramide pathway in human leukemias

Woo Jae Kim, Ross A. Okimoto, Louise E. Purton, Meagan Goodwin, Sara M. Haserlat, Farshid Dayyani, David A. Sweetser, Andrea I. McClatchey, Olivier A. Bernard, A. Thomas Look, Daphne W. Bell, David T. Scadden, and Daniel A. Haber  
*Blood* 111: 4716 (2008)

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