

# Odyssey® Publications

## In-Cell Western™ Assays

Summer 2011

Volume 2

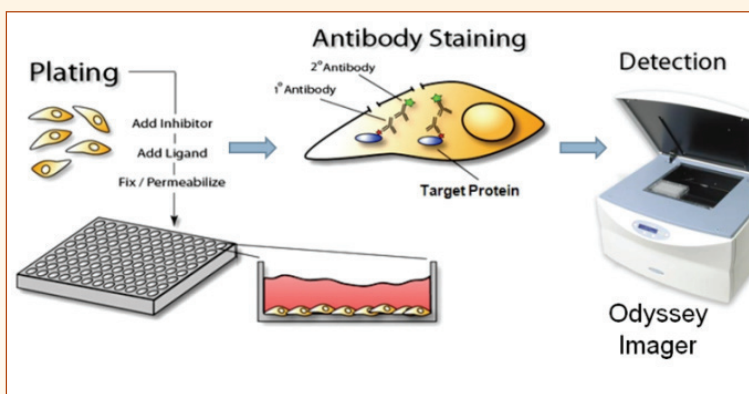
*Selected From Over 80 publications*

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### In-Cell Western Assay

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, for example, with 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 phosphospecific antibody and a nuclear stain like Draq5™. The use of two colors available on Odyssey® systems allows ratio-metric normalization across the wells of the plate and yields a quantitative assessment of the amount of phosphorylation that occurs. 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).

**Combination antibody treatment down-regulates epidermal growth factor receptor by inhibiting endosomal recycling**

Jamie B. Spangler, Jason R. Neil, Sivan Abramovitch, Yosef Yarden, Forest M. White, Douglas A. Lauffenburger, and K. Dane Wittrup  
*PNAS*, Jul 2010; 107: 13252 - 13257.

**Identification of Five Interferon-Induced Cellular Proteins That Inhibit West Nile Virus and Dengue Virus Infections**

Dong Jiang, Jessica M. Weidner, Min Qing, Xiao-Ben Pan, Haitao Guo, Chunxiao Xu, Xianchao Zhang, Alex Birk, Jinhong Chang, Pei-Yong Shi, Timothy M. Block, and Ju-Tao Guo  
*J. Virol.*, Aug 2010; 84: 8332 - 8341.

**Role of Inflammation and Insulin Resistance in Endothelial Progenitor Cell Dysfunction**

Cyrus V. Desouza, Frederick G. Hamel, Keshore Bidasee, and Kelly O'Connell  
*Diabetes*, Apr 2011; 60: 1286 - 1294.

**Biochemical, Cellular, and Anti-Inflammatory Properties of a Potent, Selective, Orally Bioavailable Benzamide Inhibitor of Rho Kinase Activity**

Lakshman E. Rajagopalan, Michael S. Davies, Larry E. Kahn, Christine M. Kornmeier, Hideaki Shimada, Toni A. Steiner, Ben S. Zweifel, Jay M. Wendling, Maria A. Payne, Richard F. Loeffler, Brenda L. Case, Monica B. Norton, Mihir D. Parikh, Olga V. Nemirovskiy, Robert J. Mourey, Jaime L. Masferrer, Thomas P. Misko, and Stephen A. Kolodziej  
*J. Pharmacol. Exp. Ther.*, Jun 2010; 333: 707 - 716.

**Selective and Potent Raf Inhibitors Paradoxically Stimulate Normal Cell Proliferation and Tumor Growth**

Josette Carnahan, Pedro J. Beltran, Carol Babij, Quynh Le, Mark J. Rose, Steven Vonderfecht, Joseph L. Kim, Adrian L. Smith, Karthik Nagapudi, Martin A. Broome, Manory Fernando, Hue Kha, Brian Belmontes, Robert Radinsky, Richard Kendall, and Teresa L. Burgess  
*Mol. Cancer Ther.*, Aug 2010; 9: 2399 - 2410.

**Facilitating Akt Clearance via Manipulation of Hsp70 Activity and Levels**

John Koren, III, Umesh K. Jinwal, Ying Jin, John O'Leary, Jeff R. Jones, Amelia G. Johnson, Laura J. Blair, Jose F. Abisambra, Lyra Chang, Yoshinari Miyata, Anna M. Cheng, Jianping Guo, Jin Q. Cheng, Jason E. Gestwicki, and Chad A. Dickey  
*J. Biol. Chem.*, Jan 2010; 285: 2498 - 2505.

**SH2 Domains Recognize Contextual Peptide Sequence Information to Determine Selectivity**

Bernard A. Liu, Karl Jablonowski, Eshana E. Shah, Brett W. Engelmann, Richard B. Jones, and Piers D. Nash  
*Mol. Cell. Proteomics*, Nov 2010; 9: 2391 - 2404.

**Regulation of Tumor Necrosis Factor-Like Weak Inducer of Apoptosis Receptor Protein (TWEAKR) Expression by Kaposi's Sarcoma-Associated Herpesvirus MicroRNA Prevents TWEAK-Induced Apoptosis and Inflammatory Cytokine Expression**

Johanna R. Abend, Thomas Uldrick, and Joseph M. Ziegelbauer  
*J. Virol.*, Dec 2010; 84: 12139 - 12151.

**A dual role for EDEM1 in the processing of rod opsin**

Maria Kosmaoglou, Naheed Kanuga, Mònica Aguilà, Pere Garriga, and Michael E. Cheetham  
*J. Cell Sci.*, Dec 2009; 122: 4465 - 4472.

**Agonist-Biased Signaling via Proteinase Activated Receptor-2: Differential Activation of Calcium and Mitogen-Activated Protein Kinase Pathways**

Rithwik Ramachandran, Koichiro Mihara, Maneesh Mathur, Moulay Driss Rochdi, Michel Bouvier, Kathryn DeFea, and Morley D. Hollenberg  
*Mol. Pharmacol.*, Oct 2009; 76: 791 - 801.

**CXCR3 activation by lentivirus infection suppresses neuronal autophagy: neuroprotective effects of antiretroviral therapy**

Yu Zhu, David Vergote, Carlos Pardo, Farshid Noorbakhsh, Justin C. McArthur, Morley D. Hollenberg, Christopher M. Overall, and Christopher Power  
*FASEB J*, Sep 2009; 23: 2928 - 2941.

**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.

**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-BEZ235, 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, Dorian Fabbro, Daniela Gabriel, Marjo Simonen, Leon Murphy, Peter Finan, William Sellers, and Carlos García-Echeverría  
*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.

**Multipathway Model Enables Prediction of Kinase Inhibitor Cross-Talk Effects on Migration of Her2-Overexpressing Mammary Epithelial Cells**

Neil Kumar, Raffi Afeyan, Hyung-Do Kim, and Douglas A. Lauffenburger  
*Mol Pharmacol* 73:1668–1678, 2008

**Androgen receptor expression in prostate cancer stem cells: is there a conundrum?**

N Sharifi, EM Hurt, and WL Farrar  
*Cancer Chemother Pharmacol*, Oct 2008; 62(5): 921-3.

**Kinetics of Wnt-driven beta-catenin stabilization revealed by quantitative and temporal imaging**

RN Hannoush

*PLoS One*, Jan 2008; 3(10): e3498.

**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, Hosseinali 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, Vijayaradhi 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.

**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* 109: 560 (2007)

**Activation of Transient Receptor Potential Vanilloid Type-1 Channel Prevents Adipogenesis and Obesity**

Li Li Zhang, Dao Yan Liu, Li Qun Ma, Zhi Dan Luo, Ting Bing Cao, Jian Zhong, Zhen Cheng Yan, Li Juan Wang,

Zhi Gang Zhao, Shan Jun Zhu, Mark Schrader, Florian Thilo, Zhi Ming Zhu, and Martin Tepel

*Circ. Res.*, Apr 2007; 100: 1063 - 1070.

**A Novel Cell-Based Assay for G-Protein-Coupled Receptor-Mediated Cyclic Adenosine Monophosphate Response Element Binding Protein Phosphorylation**

Julie V. Selkirk, Lisa M. Nottebaum, Ian C. Ford, Mark Santos, Siobhan Malany, Alan C. Foster, and Sandra M. Lechner

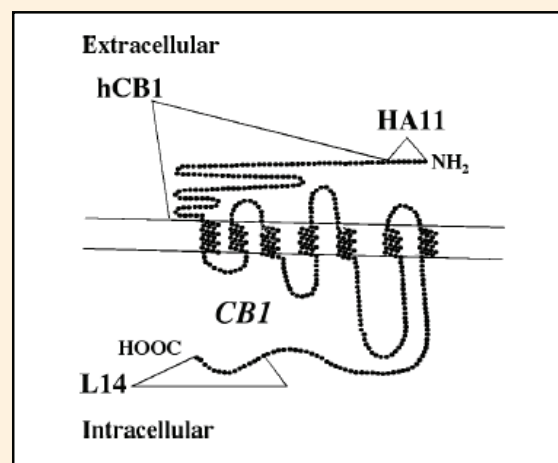
*J Biomol Screen* 11: 351 (2006)

## On-Cell Western Assay

### On-Cell Western for receptor protein trafficking

The On-Cell Western (OCW) assay allows the quantification of a protein on the surface of a cell. This is accomplished by comparing the signal from cells that are permeabilized with those that are not permeabilized. 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 et al. developed the OCW assay to study the protein trafficking processes of internalization and recycling of G-coupled protein receptors, CB1 cannabinoid receptor. The ability to quantify the amounts of protein on the surface and inside a cell is essential to understanding cellular sensitivity to extracellular ligands.



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” used 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” used to characterize total and surface tissue factor on mononuclear cells in response to LPS.
- Gonzalez-Gronow et al. employed the OCW to quantitate knockdown of the glucose regulated protein 78 (GBP78) expression on the cell surface following transfection of specific siRNAs.
- Markovic et al. used the assay to quantify the loss of the Clathrin heavy chain (CRH-R2) receptors from the cell surface after agonist stimulation.

### Loss of the C Terminus of Melanocortin Receptor 2 (MC2R) Results in Impaired Cell Surface Expression and ACTH Insensitivity

Andrea Hirsch, Eirini Meimaridou, Monica Fernandez-Cancio, Amit V. Pandey, María Clemente, Laura Audi, Adrian J. L. Clark, and Christa E. Flück  
*J. Clin. Endocrinol. Metab.*, Jan 2011; 96: E65 - E72.

### AKAP79/150 Impacts Intrinsic Excitability of Hippocampal Neurons through Phospho-Regulation of A-type K<sup>+</sup> Channel Trafficking

Lin Lin, Wei Sun, Faith Kung, Mark L. Dell'Acqua, and Dax A. Hoffman  
*J. Neurosci.*, Jan 2011; 31: 1323 - 1332.

### Targeting of the Orphan Receptor GPR35 by Pamoic Acid: A Potent Activator of Extracellular Signal-Regulated Kinase and $\beta$ -Arrestin2 with Antinociceptive Activity

Pingwei Zhao, Haleli Sharir, Ankur Kapur, Alan Cowan, Ellen B. Geller, Martin W. Adler, Herbert H. Seltzman, Patricia H. Reggio, Susanne Heynen-Genel, Michelle Sauer, Thomas D.Y. Chung, Yushi Bai, Wei Chen, Marc G. Caron, Larry S. Barak, and Mary E. Abood  
*Mol. Pharmacol.*, Oct 2010; 78: 560 - 568.

### Modulation of endocytic trafficking and apical stability of CFTR in primary human airway epithelial cultures

Deborah M. Cholon, Wanda K. O'Neal, Scott H. Randell, John R. Riordan, and Martina Gentsch  
*Am J Physiol Lung Cell Mol Physiol*, Mar 2010; 298: L304 - L314.

**Atypical Responsiveness of the Orphan Receptor GPR55 to Cannabinoid Ligands**

Ankur Kapur, Pingwei Zhao, Haleli Sharir, Yushi Bai, Marc G. Caron, Larry S. Barak, and Mary E. Abood  
*J. Biol. Chem.*, Oct 2009; 284: 29817 - 29827.

**MRAP and MRAP2 are bidirectional regulators of the melanocortin receptor family**

Li F. Chan, Tom R. Webb, Teng-Teng Chung, Eirini Meimaridou, Sadani N. Cooray, Leonardo Guasti, J. Paul Chapple, Michaela Egertová, Maurice R. Elphick, Michael E. Cheetham, Louise A. Metherell, and Adrian J. L. Clark  
*PNAS*, Apr 2009; 106: 6146 - 6151.

**Ligation of cancer cell surface GRP78 with antibodies directed against its COOH-terminal domain up-regulates p53 activity and promotes apoptosis**

Uma Kant Misra, Yvonne Mowery, Steven Kaczowka, and Salvatore Vincent Pizzo  
*Mol. Cancer Ther.*, May 2009; 8: 1350 - 1362.

**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. Osterud  
*Journal of Thrombosis and Haemostasis*, 4: 614–620 (2006)

**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)

**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)

**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 Punna, 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 Gentsch, 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 Assay

Delisle et al. has even 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 exprssion 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)

### **Microfluidic Cell Culture and Its Application in High-Throughput Drug Screening: Cardiotoxicity Assay for hERG Channels**

Xiaojing Su, Edmond W. K. Young, Heather A. S. Underkofler, Timothy J. Kamp, Craig T. January, and David J. Beebe  
*J Biomol Screen*, Jan 2011; 16: 101 - 111.

### **Loss-of-function mutation of the *SCN3B*-encoded sodium channel $\beta$ 3 subunit associated with a case of idiopathic ventricular fibrillation**

Carmen R. Valdivia, Argelia Medeiros-Domingo, Bin Ye, Win-Kuang Shen, Timothy J. Algiers, Michael J. Ackerman, and Jonathan C. Makielski  
*Cardiovasc Res*, Jun 2010; 86: 392 - 400.

## Cell Proliferation Assay

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 in a single color ICW. Widberg et al. validated the SYTO 60 assay against direct cell counts and showed a strong positive correlation with cell number ( $r^2 = 0.9948$ ).

### **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)

### **Modeling oncogene addiction using RNA interference**

S. Michael Rothenberg, Jeffrey A. Engelman, Sheila Le, David J. Riese, II, Daniel A. Haber, and Jeffrey Settleman  
*PNAS*, Aug 2008; 105: 12480 - 12484.

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