A new way to study the PI3-kinase signaling pathway using the AKT isoforms 1, 2 and 3

Validated, robust and convenient HCS Reagent Kits

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High-content image analysis is a powerful tool for examining critical molecular events of a population on a per-cell basis. Reagent kits for high-content screening (HCS) provide an easy-to-use format for measurement of target proteins that are critical to important biological pathways. Thermo Scientific Cellomics* HCS Reagents Kits enable the study of cytotoxicity and apoptosis, inflammation and cell stress, genotoxicity, DNA damage and repair, cell signaling and transcription factors, cell cycle and proliferation, and morphology and phenotypic changes. In addition, Thermo Scientific Redistribution* Technology provides for expression and measurement of such targets via green fluorescent protein (GFP) cell lines. The Thermo Scientific Cellomics AKT Activation Kit, which is a recent addition to the product line, was developed for the study of the PI3-kinase signaling pathway using the AKT 1, 2 and 3 isoforms.

AKT (PKB) is a central kinase involved in growth factor signaling, glucose metabolism and homeostasis. After signal transduction by growth factors, cytokines, mitogens or hormones, PI3 kinase phosphorylates AKT at serine473 and threonine308 at the cellular membrane. Once active, AKT translocates to other subcellular locations to phosphorylate target proteins involved in metabolism, protein synthesis, apoptosis, transcription factor regulation and the cell cycle. Alterations in AKT signaling lead to uncontrolled cell proliferation, and genetic mutations in the PI3-kinase signaling pathway are prominent in colon, breast and prostate cancers.

AKT is a challenging target for HCS because of its constitutive cellular expression, and its rapid membrane translocation and cytosolic localization. The AKT Activation Kit uses modified media to reduce endogenous levels of phospho-AKT and Whole Cell Stain Green to capture intensity differences at the cell membrane and in the entire cytosol. This kit contains a rabbit polyclonal phospho-AKT antibody, a goat anti-rabbit secondary antibody conjugated to the DyLight* 649 Fluorophore, Whole Cell Stain Green, and other reagents and buffers required for immunofluorescence detection of phospho-AKT.

AKT activation was measured using IGF-1 treated 3T3 L1 preadipocytes and the assay was optimized with the Thermo Scientific Cellomics ArrayScan* HCS Reader and Morphology Explorer BioApplication Software Module (Figure 1). In this application, many cell features, including texture measurements and intensity, are available for quantitation. After stimulation with IGF-1, AKT is phosphorylated, resulting in activation and movement of AKT to the membrane periphery as well as to other subcellular locations in the cytoplasm. AKT activation is measured using total cellular fluorescent intensity or changes in intracellular texture (including intracellular spots) (Figure 2). Cells labeled by this kit may also be imaged using fluorescence microscopy or confocal microscopy.

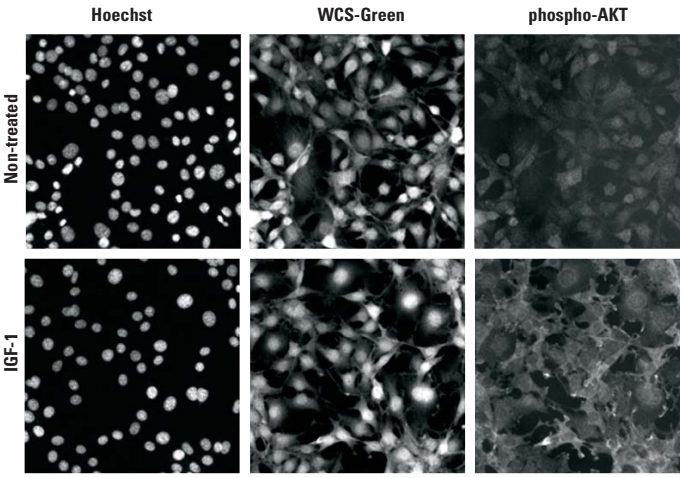

Figure 1. AKT Activation using IGF-1. 3T3 L1 cells were growth factor-deprived overnight and subsequently treated with 200 ng/ml IGF-1 for 10 minutes. Cells were stained according to the optimized procedure with phospho-AKT primary antibody, DyLight 649 Goat anti-Rabbit Secondary Antibody, Whole Cell Stain Green and Hoechst 33342 dye. Phosphorylated AKT translocates to the cytoplasm and membrane periphery upon activation. The top row represents non-treated cells imaged with different individual fluorescence channels; the bottom row represents cells treated with IGF-1.

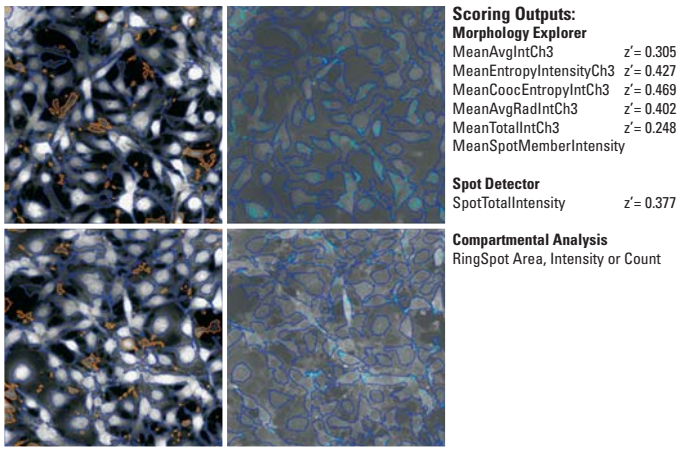

Figure 2. Data output parameters using the Thermo Scientific Cellomics ArrayScan* Instrument and Whole Cell Stain (WCS) Green. Because most algorithms are based on circular objects for identification, much of the cytosolic area for this assay would be excluded from quantitation using Hoechst for object identification. Whole Cell Stain Green captures AKT activation at the membrane and in the cytoplasm, allowing quantitation of AKT using many different output parameters (various BioApplications, Output Features and Z' values are indicated). Therefore, the use of the Whole Cell Stain enhances quantitation of phospho-AKT in the cytosol.

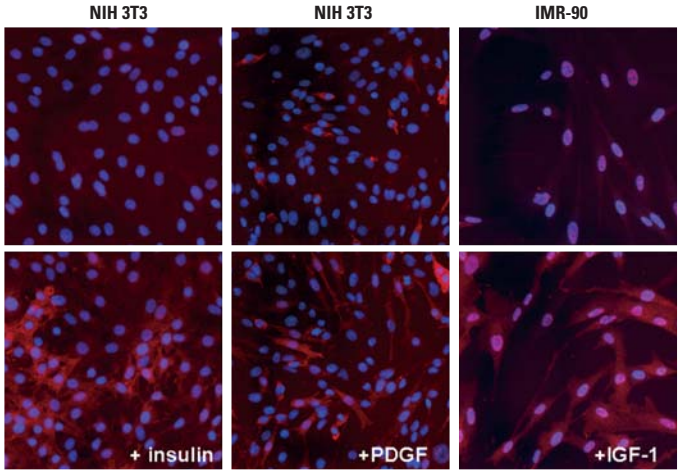

Figure 3. AKT activation in NIH 3T3 and IMR-90 fibroblasts were serum-starved overnight before treatment with insulin (30 minutes), IGF-1 (10 minutes) or PDGF (15 minutes). Cells were stained according to the kit procedure with Hoechst, Whole Cell Stain Green (not shown), pAKT antibody and DyLight 649 Goat anti-Rabbit Secondary Antibody. The top row represents merged Hoechst and AKT images in non-treated cells; the bottom row represents merged images from treated cells.

Performance robustness

The AKT Activation Kits have been tested in several cell types with different stimuli (Figure 3). Time course and dose response data are in Figure 4.

Assay robustness was ascertained by determining the Z´ for the total or average intensity in the cytoplasm in non-treated cells (min, 0 ng/ml) and cells treated with IGF-1 (max, 200 ng/ml) for 10 minutes. The mean ± SD of the Z´ was determined from three plates of 3T3 L1 cells that were treated identically and was 0.39 ± 0.06, with a coefficient of variation of 2.5 % for phospho-AKT. The assay performance using these kits was robust when compounds were added in DMSO up to a maximum concentration of 1% DMSO.

General References

1. Zhang, J.H., *et al.* (1999). A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* **4**, 67-73.
2. Taylor, D.L., *et al.* (2007). High content screening: A powerful approach to systems cell biology and drug discovery. *Method Mol Biol* **356**, Humana Press, Totowa, N.J.
3. Cantley, L.C. (2002). The phosphoinositide 3-kinase pathway. *Science* **296**, 1655-1657.
4. Du, K. and Tsichlis, P.N. (2005). Regulation of the AKT kinase by interacting proteins. *Oncogene* **24**, 7401-7409.
5. Toker, A. and Yoeli-Lerner, M. (2006). Akt signaling and cancer: Surviving but not moving on. *Cancer Res.* **66**, 3963-3966.

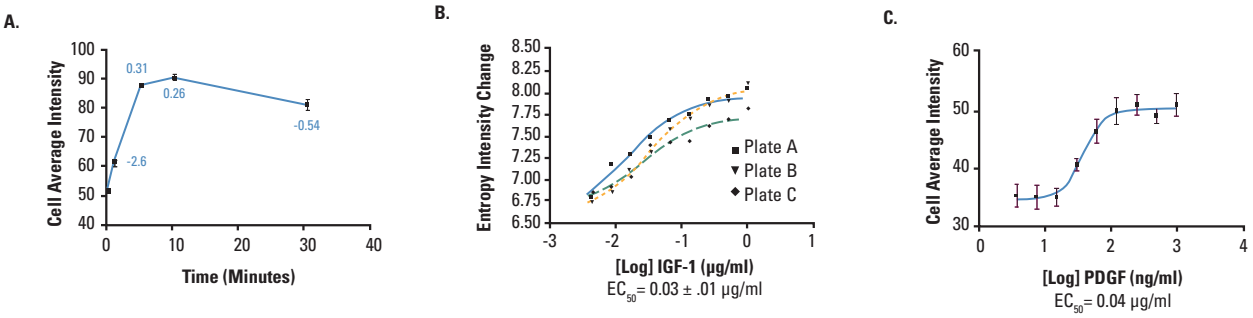

Figure 4A. AKT time-course and dose response curves. 3T3 L1 cells were challenged with 200 ng/ml IGF-1 for the indicated times. AKT activation was measured using the average cell intensity. Each point represents data from 32 wells. Numbers in red indicate the Z' value at that time point. AKT phosphorylation is rapid and diminishes quickly. **B and C**: Dose Response Curves. 3T3 L1 or NIH 3T3 cells were growth factor-deprived overnight and then challenged with the indicated concentrations of IGF-1 (3T3 L1: 10 minutes) or PDGF (NIH 3T3: 15 minutes). EC50 values are indicated. **B**. The lines represent three independent plates and each point represents data from eight wells in three independent plates. **C**. Each point represents data from eight wells from a single plate.

Ordering Information:

Product #	Description	Pkg. Size
8404101	Phospho-AKT Activation Kit	1 x 96
8404102	Phospho-AKT Activation Kit	5 x 96
Kits for Inflammation & Stress Cells		
K01-0001-1	NFkB Activation HCS Reagent Kit	5 x 96
R01-0502-1	NFkB Activation HCS Reagent Kit	50 x 96
8400301	NFkB and Green c-Jun Activation Kit	1 x 96
8400302	NFkB and c-Jun Activation Kit	5 x 96
8400401	NFkB and c-Jun Activation Kit	1 x 96
8400402	Orange NFkB and c-Jun Activation Kit	5 x 96
K01-0003-1	c-Jun Activation HCS Reagent Kit	5 x 96
R01-0508-1	c-Jun Activation HCS Reagent Kit	50 x 96
K01-0011-1	NFAT Activation HCS Reagent Kit	5 x 96
R01-0515-1	NFAT-1 Activation HCS Reagent Kit	50 x 96
K01-0004-1	p38 MAPK Activation HCS Reagent Kit	5 x 96
R01-0507-1	p38 MAPK Activation HCS Reagent Kit	50 x 96
8401001	Oxidative Stress Kit 1	1 x 96
8401002	Oxidative Stress Kit 1	5 x 96
K01-0002-1	STAT1 Activation HCS Reagent Kit	5 x 96
R01-0503-1	STAT1 Activation HCS Reagent Kit	50 x 96
K01-0007-1	ERK MAPK (Green) Activation	5 x 96

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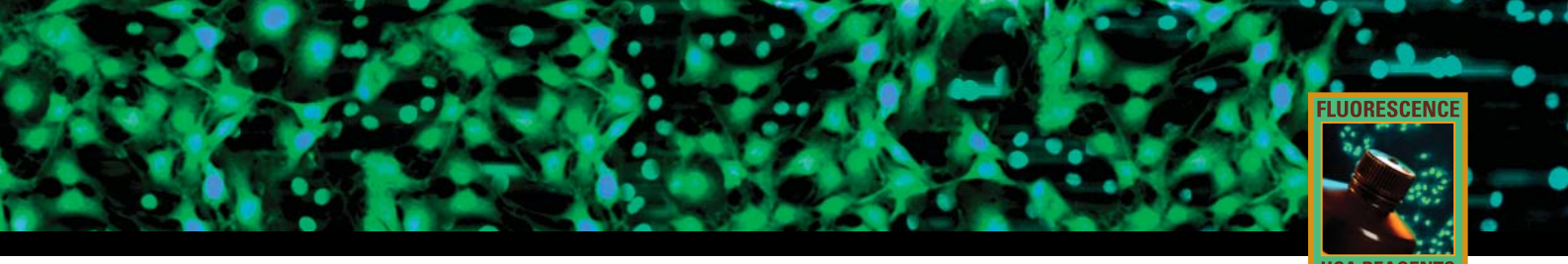

R01-0509-1	ERK MAPK (Green) Activation	50 x 96
K01-0005-1	STAT2 Activation HCS Reagent Kit	5 x 96
R01-0504-1	STAT2 Activation HCS Reagent Kit	50 x 96
K01-0008-1	STAT3 Activation HCS Reagent Kit	5 x 96
R01-0505-1	STAT3 Activation HCS Reagent Kit	50 x 96
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R02-0005-1	Cell Motility HCS Reagent	50 x 96
K01-0010-1	ATF-2 Activation Evaluation HCS Reagent Kit	5 x 96
R01-0514-1	ATF-2 Activation Evaluation HCS Reagent Kit	50 x 96
8403801	iNOS Activation Kit	1 x 96
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8403702	COX 2 Activation Kit	5 x 96
8403901	CHOP/GADD153 Detection Kit	1 x 96
8403902	CHOP/GADD153 Detection Kit	5 x 96
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8404002	JNK Activation Kit	5 x 96
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8404502	Phospho-Rb Activation Kit	5 x 96
8404801	Phospho-PLK1 Activation Kit	1 x 96
8404802	Phospho-PLK1 Activation Kit	5 x 96
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R01-0510-1	Mitotic Index HCS Reagent Kit	50 x 96
K11-0001-1	Micronucleus HCS Reagent Kit	5 x 96
8400501	Phospho-p53 and p53 Detection Kit	1 x 96
8400502	Phospho-p53 and p53 Detection Kit	5 x 96
8400601	p53 and p21 Detection Kit	1 x 96
8400602	p53 and p21 Detection Kit	5 x 96
8400701	Phospho-p53 Detection Kit	1 x 96
8400702	Phospho-p53 Detection Kit	5 x 96
8400801	p53 Detection Kit	1 x 96
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8400901	p21 Detection Kit	1 x 96
8400902	p21 Detection Kit	5 x 96
8401801	MDM2 and p53 Detection Kit – Orange p53 and Green MDM2	1 x 96
8401802	MDM2 and p53 Detection Kit – Orange p53 and Green MDM2	5 x 96
8401901	MDM2 Detection Kit – Orange MDM2	1 x 96
8401902	MDM2 Detection Kit – Orange MDM2	5 x 96
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8402902	Phospho H2AX Activation Kit*	5 x 96
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K02-0002-1	Multiparameter Cytotoxicity I	5 x 96
R02-0006-1	Multiparameter Cytotoxicity I	50 x 96
K04-0001-1	Apoptosis I (multiparameter)	5 x 96
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8400001	Multiparameter Cytotoxicity II Kit Containing both Lysosome and Mitochondrial Probes	1 x 96
8400002	Multiparameter Cytotoxicity II Kit Containing both Lysosome and Mitochondrial Probes	5 x 96
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8400102	Multiparameter Cytotoxicity II Kit Containing Lysosome Probe	5 x 96
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8401102	BrdU and Ki67 Cell Proliferation Kit	5 x 96
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8401202	BrdU Cell Proliferation Kit	5 x 96
8401301	Ki67 Cell Proliferation Kit	1 x 96
8401302	Ki67 Cell Proliferation Kit	5 x 96

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