SARTORIUS

Simplifying Progress

Visualization and High-throughput Quantification of Akt Activity in Live-Cell Neuroinflammatory Models

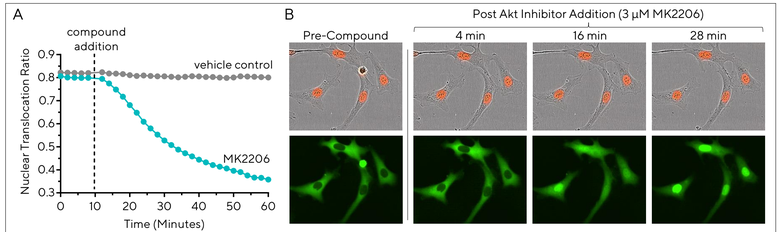
J. Trigg[1*], G. Lovell[1], A. Ward[1], J. Rauch[2], G. Filonov[2], S. L. Alcantara[1], and T. Dale[1]

Sartorius Corporation; [1]Royston UK, [2]Ann Arbor MI
*Corresponding author: Jasmine.Trigg@Sartorius.com

Introduction

- Chronic neuroinflammatory states are associated with the development of several neurodegenerative diseases. The PI3K/Akt signaling pathway has been implicated in these disease processes and holds therapeutic promise as a target to modulate neuroinflammatory responses.
- Here we demonstrate a robust *in vitro* assay to assess dynamic changes in Akt activity in real-time.
- To monitor Akt we used astrocytic and immune cells stably expressing the Incucyte® Kinase Akt Green/Red biosensor, a genetically-encoded fluorescent kinase translocation reporter whose subcellular localization is dependent on phosphorylation by Akt.
- We investigated the effects of inhibitors targeting the PI3K/Akt pathway and observed differential time- and concentration-dependent responses. We also examined the effects of immunocompetent cell activation on Akt using LPS stimulation and inflammatory cytokines.
- These data exemplify the Incucyte® Kinase Akt Assay as a powerful live-cell approach for assessing Akt activity in neuroinflammatory models and its amenability to screening of therapeutic candidates.

Live-cell imaging of Akt kinase activity

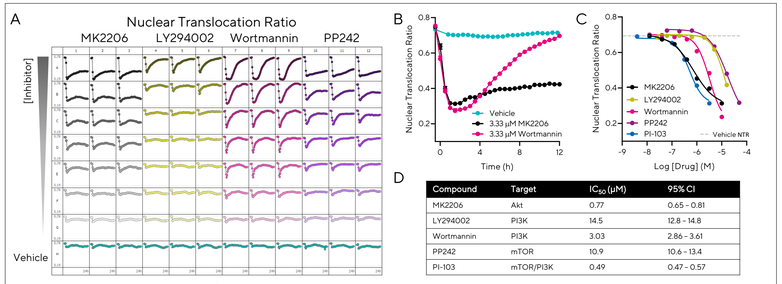


- SK-OV-3 cells stably expressing the Incucyte® Kinase Akt Green/Red biosensor were treated with selective Akt inhibitor MK2206. Phase and fluorescent images were acquired using the Incucyte® Live-Cell Analysis System and analyzed using integrated software.

A) Time course shows a kinetic decrease in Nuclear Translocation Ratio (NTR) compared to vehicle indicating Akt inhibition.

B) Image panel shows phase and red channels (top) and green fluorescence channel (bottom), demonstrating translocation of the green fluorescent biosensor from the cytoplasm to the nucleus following MK2206 treatment.

Direct and indirect inhibitory compound effects on Akt activity



D

Compound	Target	IC_{50} (µM)	95% CI
MK2206	Akt	0.77	0.65 – 0.81
LY294002	PI3K	14.5	12.8 – 14.8
Wortmannin	PI3K	3.03	2.86 – 3.61
PP242	mTOR	10.9	10.6 – 13.4
PI-103	mTOR/PI3K	0.49	0.47 – 0.57

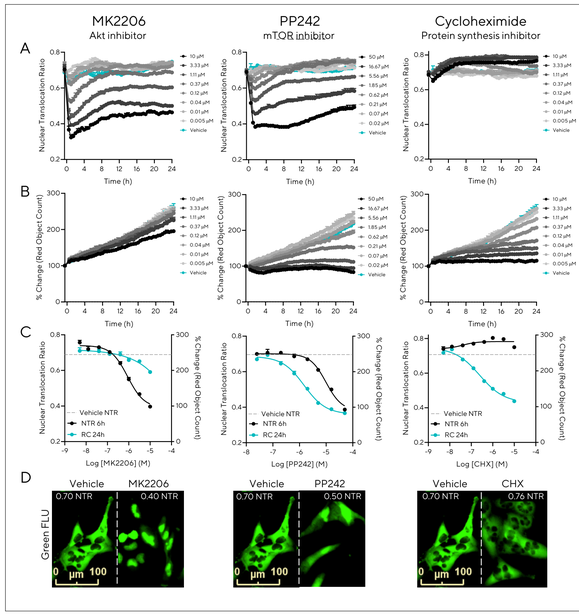
- T98G cells expressing the Incucyte® Kinase Akt biosensor were treated with inhibitors targeting Akt, PI3K, and mTOR.

A) Microplate graph displays NTR response over 24h with a concentration-dependent decrease observed for all four compounds.

B) Time course exemplifies varying kinetic profiles observed. Allosteric Akt inhibitor MK2206 induced sustained inhibition, whist PI3K inhibitor Wortmannin showed rapid inhibition followed by recovery to baseline indicating Akt reactivation.

C) Transformation of data at 6h post-treatment indicates increased potency for Akt or dual mTOR/PI3K inhibitors compared to inhibitors of mTOR or PI3K alone, with IC_{50} values shown in table (D).

Concurrent quantification of Akt activity and cell proliferation



- T98G astrocytes expressing Incucyte® Kinase Akt biosensor were treated with 3 inhibitors. NTR (Akt activity) and Red Object Count (RC; proliferation) were monitored concurrently.

A) NTR time courses show concentration-dependent inhibition of Akt activity by MK2206 and PP242, with slight activation for Cycloheximide (CHX).

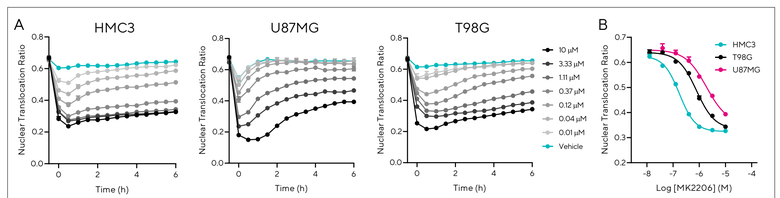
B) RC time courses reveal concentration-dependent inhibition of proliferation for PP242 and CHX but not MK2206.

C) IC_{50} graphs of NTR and RC (6h or 24h post-treatment, respectively) are shown.

D) Green fluorescence images (6h post-treatment) show nuclear translocation for MK2206 (10 µM) and PP242 (16.7 µM) but not CHX (10 µM) compared to vehicle.

- Data reveals varying effects of inhibitors with different mechanisms of action on Akt activity and cell proliferation.

Comparison of Akt inhibitor MK2206 across neural cell types



- HMC3 microglia, and U87MG and T98G astrocytes expressing the Incucyte® Kinase Akt biosensor were treated with selective Akt inhibitor MK2206.

A) Time courses exhibit a kinetic concentration-dependent decrease in NTR, indicating inhibition of Akt activity across all cell lines.

B) MK2206 showed increased potency for HMC3, compared to T98G or U87MG (IC_{50} values of 0.17, 95% CI [0.15, 0.20], 0.73 95% CI [0.68, 0.83], and 1.29 µM 95% CI [1.06, 1.59], respectively at 6h post-treatment).

Incucyte® Live-Cell Imaging and Analysis Solutions

Incucyte® Live-Cell Analysis System

A fully automated HD phase-contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

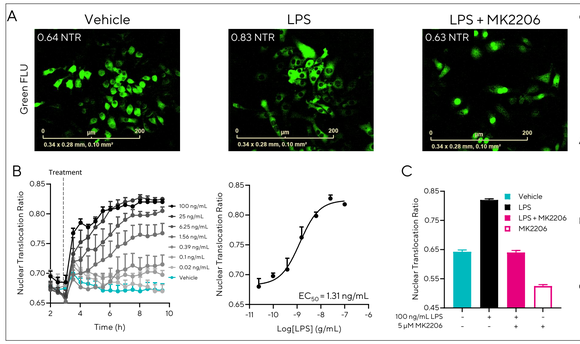
Incucyte® Software

Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

Incucyte® Reagents and Consumables

A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

Activation of Akt in LPS-stimulated immune cells



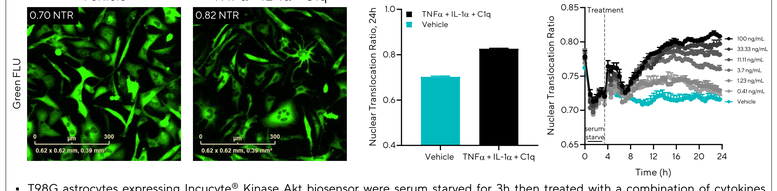
- RAW264.7 monocytes expressing Incucyte® Kinase Akt biosensor were serum starved for 3h to inactivate Akt then treated with LPS and/or Akt inhibitor MK2206. Images and data shown at 6h post-treatment.

A) Green fluorescence images show cytoplasmic translocation for LPS (100 ng/mL) compared to vehicle which is blocked by MK2206 (5 µM).

B) Graphs show LPS induced a kinetic concentration-dependent increase in NTR indicating Akt activation.

C) Basal and LPS stimulation-induced Akt activity are reduced in the presence of Akt inhibitor MK2206.

Inflammatory effect of cytokines on Akt activity



- T98G astrocytes expressing Incucyte® Kinase Akt biosensor were serum starved for 3h then treated with a combination of cytokines known to induce reactivity (TNFα, IL-1 α, and C1q) or a concentration range of inflammatory cytokine TNFα and monitored for 24h.

A) Green fluorescence images (6h post-treatment) are suggestive of cytoplasmic translocation for the cytokine cocktail compared to vehicle.

B) Quantification at 24h reveals an increase in NTR (17.8%) for the cytokine cocktail, indicating modest Akt activation.

C) TNFα induced a concentration-dependent increase in NTR over time that peaked by 24h.

- Preliminary data indicates potential role of Akt in inflammatory cytokine-induced astrocytic activation, although further studies are required.

Data presented as mean ± SEM, 3 – 6 replicates