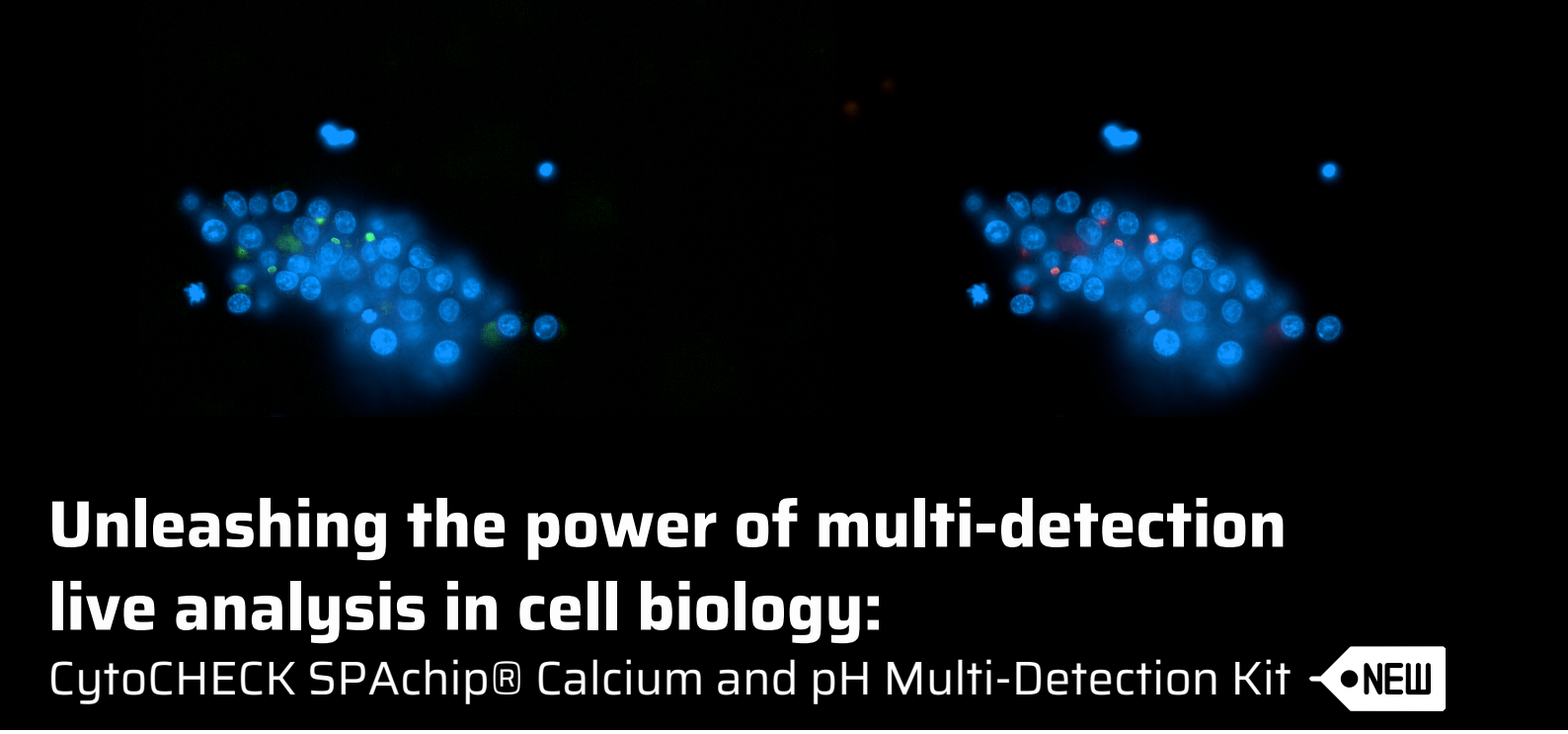

Unleashing the power of multi-detection live analysis in cell biology:

CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit NEW

A comprehensive study of the living single-cell physiology is crucial to better understand cell's ability to initiate differentiation and proliferation and to maintain its metabolic state. In this sense, the effects of changes of Ca^{2+} and pH ions are important to cellular functions.

CytoCHECK SPAchip® assay kits are composed of fluorescently labeled silicon microparticles -SPAchips®- that can be internalized in the cytosol of cultured cells. The **CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit** is a two-sensor-in-one reagent that enables simultaneous measurement of cytosolic calcium ion and intracellular and extracellular pH levels through changes in fluorescence intensity.

Now, you are not limited to only monitoring one single physiological marker per sample: the **CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit** simplifies cell-based assay by using one single device to quantify pH and calcium variations inside individual living cells without any perturbation of their physiological state over long periods of time.

a4cell nanodevices

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APPLICATIONS

01 FUNCTIONAL CELLULAR STUDIES

Long-term monitoring of pH and Ca^{2+} provides insights into cell dynamics and signalling pathways analysis.

02 CELL VIABILITY AND CYTOTOXICITY

Better understanding of early processes of apoptosis and cell health and toxicity in response to any compound treatment.

03 CELL DIFFERENTIATION PROCESSES

Changes of pH and Ca^{2+} affect cellular proliferation and differentiation and its underlying mechanisms.

04 METABOLIC PATHWAYS

Changes in pH and Ca^{2+} can be associated with different pathological conditions and certain metabolic disorders.

05 MITOCHONDRIA AND CELL HEALTH

pH and calcium are interplayed hallmarks of mitochondria and cell health and homeostasis (1,2,3,4).

06 DRUG EFFICACY TESTING

Tracking pH and Ca^{2+} variations to assess efficiency and specificity of drug delivery.

07 STUDY OF ENZYMATIC REACTIONS

Key metabolic enzymes operate within narrow pH and calcium ranges. Accurate monitoring ensures that these biological catalysts can perform optimally.

2-sensors-in-one SPAchip: tracking pH and calcium changes in SH-SY5Y human neuroblastoma cells

In neural cells, **extracellular pH** is crucial as it can interfere neuronal activity and the modulation of diverse types of neuronal ion channels (5,6). At the same time, **intracellular proton fluxes** are essential for energetic metabolism and cell physiology. Additionally, **calcium ions** play a vital role in the functioning of neural cells since they are involved in a wide range of physiological processes, including neurotransmitter release.

Here, we analyzed pH and Ca^{2+} changes in **SH-SY5Y human neuroblastoma cells** using **CytoCHECK SPAchip® Calcium and pH Multi-Detection kit**. Firstly, we observed internalized multi-detection SPAchips in both green and red fluorescence channels. Secondly, calcium concentration analysis in SH-SY5Y cell line was performed, observing a higher calcium and slightly lower calcium concentrations (compared with basal control) upon DOXO and BAPTA treatments, respectively (Fig. 1). Thirdly, we quantified pH measurement for an unknown sample (Fig. 2) normalizing against a pH curve with different pH conditions inside neuroblastoma cells.

CytoCHECK SPAchip® Calcium and pH Multi-Detection kit allows to easily detect, with a single SPAchip, **pH and calcium dynamics inside single living cells** (e.g. SH-SY5Y human neuroblastoma cells; Fig. 3) without causing cytotoxicity over long periods of time. Thus, our SPAchip biosensors facilitate accurately monitor transitions of physiologycal markers and cell health states, in continuous kinetics and end-point assays; saving time and costs, maximizing the usage of precious samples, diminishing variability and extracting more reliable correlations.

Experiment setup:

- **Cell line:**
  - **SH-SY5Y** (epithelial/neuronal; neuroblastoma cells).
  - Any other cell type of your choice.
- **Fluorescent dyes:**
  - Nuclei staining.
  - CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit.
- **Positive and negative controls**
- **Measurements:**
  - Total nuclei count.
  - pH measurements.
  - Calcium measurements.

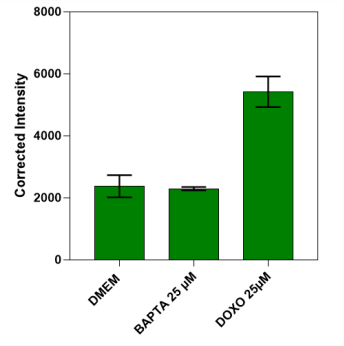

Figure 1: CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit in green for calcium measurements. Graph showing corrected fluorescence intensity values of intracellular readouts in basal conditions (with DMEM), BAPTA 25 µM, and DOXO 25 µM. Corrected fluorescence intensity slightly decreased in cells incubated with BAPTA 25 µM compared with basal control. Corrected fluorescence intensity severely increased in cells incubated with Doxorubicin 25 µM compared with basal control. Bars represent mean values for each condition and error bars correspond to SD.

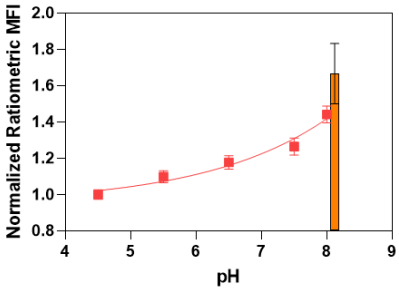

Figure 2: CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit in red for pH measurements. Graph showing ratiometric normalized fluorescence intensity values at different pH conditions using commercial calibrators inside SH-SY5Y cells (neuroblastoma cells). Ratiometric values were obtained by dividing λemi2=707 / λemi1=610 emission signals in HCS-Operetta equipment with the excitation in the range λexc=546/15 nm. Red line shows intracellular SPAchip® pH values. Orange bar shows data for an "unknown sample". Mean values for each condition were represented and error bars correspond to SD.

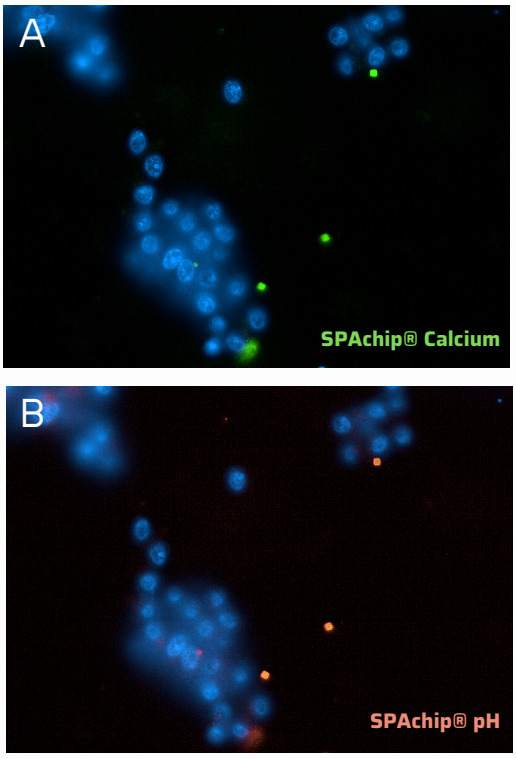

Figure 3: SH-SY5Y cell line (neuroblastoma cells) with nuclei stained in blue and CytoCHECK SPAchip® Calcium and pH Multi-Detection Kit in green and red. Images **A)** and **B)** correspond to the **same field** but different fluorescence channels. Representative images of SH-SY5Y cells with internalized multiplex SPAchips **A)** excitating at 488 nm and emitting at 520 nm and **B)** excitating at 546 nm and collecting emission at 610 and 707 nm.

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