CytoCHEK SPAchip™ pH RED Single-Detection Kit

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Key features

Product overview	**Intracellular and extracellular pH** detection. It can be used for kinetic real-time monitorization in live cells. **For long periods of time.** After resuspending SPAchips in the assay buffer, simply add it to the cells and incubate overnight to let the chips being incorporated in the cytosol. SPAchips will remain in the cytosol to monitor the evolution of pH in the cell culture.
Assay type	Live cells.
Detection method	Fluorescence.
Platform	Fluorescence microscopy, HCS/HCA platforms and flow cytometry.
Sample type	Adherent cells, suspension cells.
Applications	Cell viability, proliferation, citotoxicity, cell image acquisition.
Fluorescence Acquisition	**Ratiometric** acquisition is required for optimal performance. Excitation should be performed at **λex**: **546** nm; while emission **λem**: **610** and **707 nm**
Notes	This product is intended to be used for monitoring pH fluctuations in live cells using fluorescence microscopy or flow cytometry techniques. Platform resolution needs to be below 3 µm to identify SPAchips (20x magnification or over are suggested for microscopy applications).

Overview

CytoCHEK SPAchip® assay kits are novel **fluorescence** assays developed by A4Cell that bring together the fields of nanotechnology and cell biology. SPAchips are composed of fluorescently labeled silicon microparticles that can be internalized in the **cytosol** of cultured cells to monitor intracellular parameters for **extended periods of time**.

CytoCHECK SPAchip® pH RED Single-Detection Kit allows measurement of intracellular pH levels by changes in fluorescence intensity, which allows a more comprehensive study of the **living single-cell physiology** and maximizes the performance of most of imaging analyzers. Its signal in red makes it feasible for applications with high green autofluorescence such as organoids cultures. Moreover, due to its **ratiometric** behavior signal-to-noise ratio is increased leading to more precise curves.

Advantages

Physiologically relevant pH results: SPAchips are not toxic to the cells so it is possible measure pH in a **physiological cell state** compared to conventional assays that require much higher amounts of probe, therefore, are toxic to the cell.

Intracellular and extracellular pH measurement: Because there will be chips that do not enter the cell, it is possible to obtain an **extracellular-intracellular pH ratio** in the same assay and in a simple and fast way.

More Robust Performance: Simple protocol minimize, sample handling, reducing variability.

Improvement of experimental workflows: Only one cell culture is needed and it is possible to measure changes in fluorescence signal **for extended periods of time** (up to months).

Simplified Protocol: Assay reagents are added directly to cell culture and SPAchips will remain in the cytosol to monitor the evolution of pH levels.

Adaptable to Automation: The kit is compatible with High Content Screening (HCS) platforms and the protocol is adaptable to automation.

Relevance

Intracellular pH (pHi) is tightly regulated within cells, and many cellular functions are dependent upon proper regulation of intracellular pH. Imbalance in intracellular pH can effect many cellular functions, such as ionic homeostasis, reactive oxygen species balance, apoptosis, cell cycle, and cellular mobility. Changes of intracellular pH are also often one of the responses of cells to externally applied agents, including growth factors, hormones, and neurotransmitters.
In general, the cytoplasm tends to acidify as a result of catabolism and a negative membrane potential that drives the accumulation of H+ through cation channels and the loss of basic HCO_3^- through anion channels. Countering this acidification are intrinsic buffers and HCO_3^-, as well as distinct plasma membrane pH-regulatory transporters that finely control pH to keep it close to neutral, a level that is optimal for many protein interactions and cellular processes.

Applications

Many cell processes, such as those regulating metabolism or proliferation, are highly sensitive to pH changes. In this regard, net charge and structure of macromolecules also depend on proton concentration and maintaining intracellular proton fluxes is crucial for energetic metabolism. Therefore, the control of cytosolic pH is pivotal for the cell and the organism itself and, thus, measuring pH changes can be of importance for understanding cell physiology. Measurement of intracellular pH for alkaline conditions can offer insights of processes driving diverse signaling cascades during metabolic impairment. Cytosolic

alkalinization can be an important second messenger preceding ROS production and cytosolic Ca^{2+} fluxes during tumorigenesis and other pathological conditions.

Properties

State	Solid-phase film with embedded SPAchips		
Solubility	Soluble in assay buffer (aqueous)		
SPAchip® Quantity	~2.5 million SPAchips		
Measuring range	**pH 4.5 – 9.0**		
Storage	Store at 2-8 ºC and protected from the light.		
Shipping	Room temperature.		
Fluorescence	**λex**: **546** nm; **λem**: **610** and **707 nm**.		
Preparation time	30 minutes	**Incubation time**	Overnight

Live and Fixed-Cell Compability

Product Name	Live-Cell Staining	Fixation/Permeabilization Steps Post Live-Cell Staining	Fixed-Cell Staining
CytoCHEK SPAchip™ **pH RED** Single-Detection Kit	✓	X	X

Materials

Materials supplied	Amount	Units
Tube with sterile assay **buffer**	5 mL	1
Tube with **control** SPAchips	10 µL	1
Tube with **assay** SPAchips	~$2.5{\cdot}10^6$ SPAchips	1

Materials to be supplied by the user

Pipettes and pipette tips (1-10 µL, 2-20 µL, 20-200 µL, 50-300 µL, 200-1000 µL)

Vortex mixer

Mini-centrifuge

Reagent reservoirs

Cell culture facilities

Arrays for Cell Nanodevices S.L.
CAIT-UPM. Parque Científico y Tecnológico. Campus Montegancedo s/n
28223-Pozuelo de Alarcón (Madrid)- **info@a4cell.com**

Cell culture media (phenol red free is recommended)
Cell culture plate (p96 or p384 multiwell is recommended)

Cell imaging system (fluorescence microscope, flow cytometry or High Content Screening reader). At least 20x magnification objectives are required for quantitative analysis.

Image analysis software (Contact A4Cell Staff for support)

If quantitative analysis is required, it will be necessary:
pH calibration kit for standard curve definition (pH range: 4.5-9.0)

Handling and storage

Precautions for safe handling

Work under sterile conditions. Protect the tubes from light, especially from UV light Use in a laminar flow hood, with air supplied by an independent system. Avoid inhalation, contact with eyes, skin and clothing. Avoid the formation of dust and aerosols.

Conditions for safe storage, including any incompatibilities.

Recommended storage temperature: 2-8°C. Store in cool, well-ventilated area. Keep away from direct sunlight. Keep container tightly sealed until ready to use.

Limitations

Assay kit intended for research use only. Not for use in diagnostic procedures.
Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Imaging and Fluorescence Signal Analysis

SPAchip® kits are designed to dynamically shift fluorescence intensity values in response to intracellular changes in analyte concentration. Fluorescence intensity is measured by **exciting** the live cell sample at **546 nm** and detecting fluorescence **emission at 610 and 707 nm**. Normalization of fluorescence intensity values collected at both emission wavelengths provide a **ratiometric** behavior that allows a more precise quantification of intracellular pH. Relative intensity units depend on the instrumental setup (excitation power, detector efficiency, etc). To obtain quantitative data, a calibration curve needs to be analyzed in the same run. Data acquisition can be adapted to fluorescence microscopy and flow cytometry. Additional detection channels, such as brightfield, phase contrast, FSC or SSC, are suggested to better identify individual SPAchips.

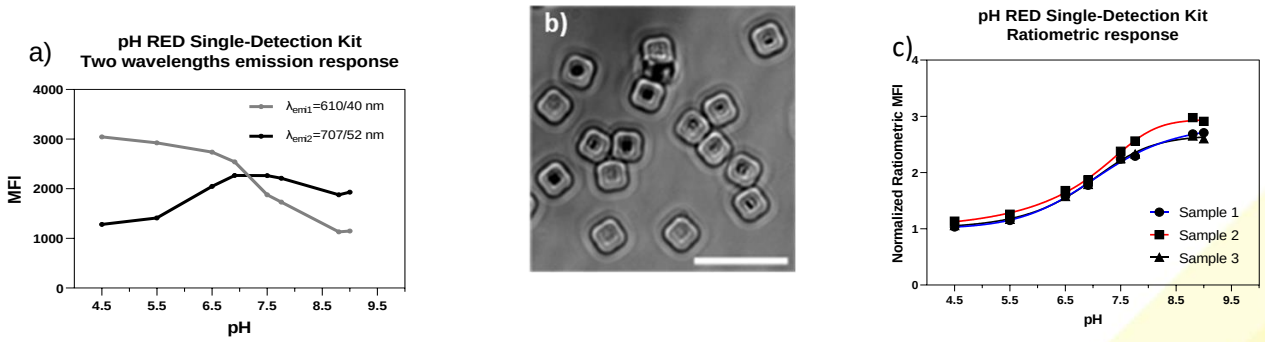

Figure 2: CytoCHECK SPAchip® RED pH Single Detection at different pH conditions using commercial calibrators. a) Graph showing emission signal at two different wavelengths for pH 4.5 – 9.0. b) Brightfield image of SPAchips® once in solution (Image from Torras et al. 2015 DOI: 10.1002/adma.201504164), Scale bar = 10 µm. c) Graph showing ratiometric normalized fluorescence intensity values at different pH. Ratiometric values were obtained by dividing λemi2/ λemi1 emission signals in HCS-Operetta equipment with the excitation in the range λexc=546/15 nm.

SPAchip® fluorescence signal quantification can be adapted to any analysis image software. Object segmentation should be performed to identify SPAchips inside cellular region to every sample. Fluorescence intensity units can be exported as a spreadsheet to plot graphical summaries of the results. Quantitative data need to be interpolated from the calibration curve values to each experiment. Normalization of results to experimental controls offers a semi-quantitative approach.

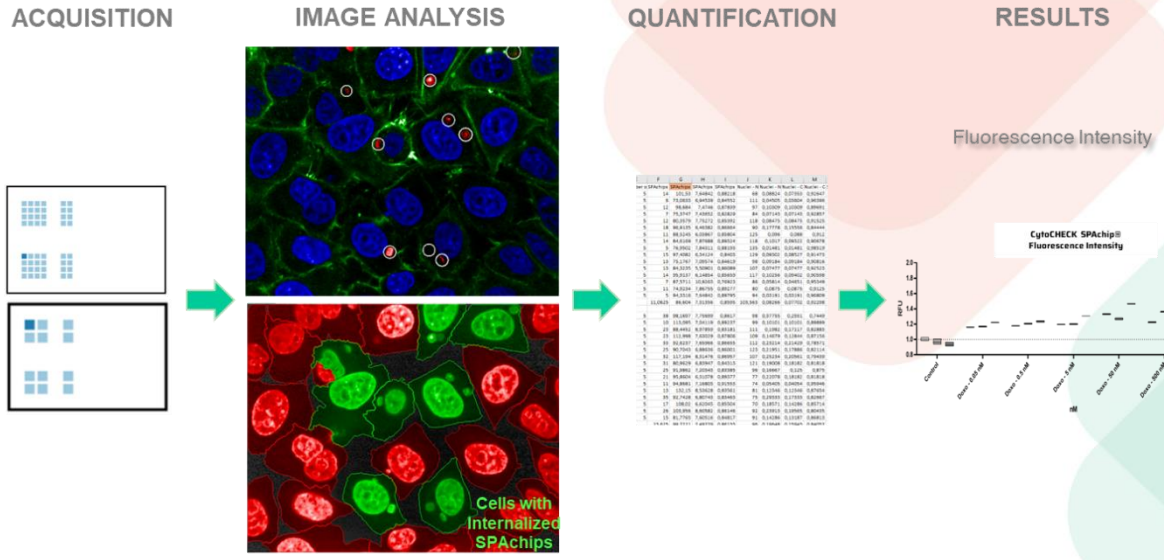

Figure 2: Image Analysis Workflow. From sample data acquisition to object identification, quantification and result presentation.

FAQs

How are SPAchips internalized?

After addition and incubation of SPAchip® solution to the culture, cells endocytose the SPAchip® in their cytosol being in direct contact with the cytosol fluid and allowing direct measurement of intracellular analytes.

How is SPAchip® uptake controlled?

SPAchip® size (3x3x1 μm^3) and geometry have been optimized to avoid cytotoxicity while yielding an optimal uptake ratio, although internalization is cell type dependent. We suggest adding a 2:1 SPAchip®:cell ratio at the measurement time point, but optimization may be required. By adding the suggested ratio to the culture, most of the cells show only 1 SPAchip® in the cell cytosol, although there can be cells which have uptaken more than 1 SPAchip®.

Are SPAchip® measurements dynamic?

SPAchip® are continuously detecting changes in the analyte levels in the cytosol offering dynamic, real-time measurements by changes in the fluorescence intensity. Compared to traditional end-point assays, SPAchip® are advantageous because preparation of multiple samples to analyze different time points become unnecessary, thus saving time and resources. Additionally, the possibility of monitoring samples over time allows to keep track of drug responses, differentiation processes or cell trajectories, which could not be precisely assessed otherwise.

What happens to SPAchip® upon cell division?

SPAchip® are solid devices, thus they do not follow cell growth over time. For this reason, upon cell division only one of the daughter cells keep the SPAchip® in its cytosol, hence SPAchip®-to-cell ratio should be designed according to the expected cell number/confluency.

Can SPAchip® devices be affected by photobleaching?

As any other fluorophore, SPAchip® can be subject of photobleaching effect. However, our devices have been optimized to suffer a mild decay in the fluorescence signal (<20%) after continuous and intensive laser exposure (1h, 13 cycles), with all calibration conditions maintaining significant differences in intensity.

Are SPAchip® compatible with other assays?

SPAchip® can be used with any other fluorescent readouts, including stainings, engineered cell lines, cell painting approaches, etc. Excitation and emission wavelengths need to be considered to avoid undesired signal overlap. Please contact A4Cell if you need support to design your panel.

Technical hints

- This kit is provided based on SPAchip® number. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical staff for further Support inquiries.
- Selected components in this kit are supplied in surplus quantity to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet.
- Valid for 6 months from the arrival date.
- Response to your inquiry within 24 hours.
- We provide support in English and Spanish.
- Multi-media technical resources to help you.

Terms and conditions

Guarantee only valid for products bought direct from A4Cell or one of our authorized distributors.