CytoCHECK SPAchip® Calcium Single-Detection Kit

Table of Contents

Key features

Product overview	Intracellular Calcium 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 Calcium 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, cell image acquisition.
Notes	This product is intended to be used for monitoring calcium 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).

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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™ Calcium Single-Detection Kit allows measurement of intracellular Calcium 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.

Advantages

Physiologically relevant Calcium results: SPAchips are not toxic to the cells, thus, making possible to measure Calcium in a physiological cell state compared to conventional assays that require much higher amounts of probe, therefore, are toxic to the cell.

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 (months).

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

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

Relevance

Calcium is essential for all living organisms. Ca^{2+} sequestration and release into and out of the cytoplasm functions as a signal for many cellular processes, such as gene expression, metabolism, proliferation, secretion, neural excitation and fertilization. The intracellular Ca^{2+} is sequestered into intracellular organelles which they provide both an internal Ca^{2+} regulation and distribution system, and a scaffold for the synthesis, targeting, and insertion of channels and receptors.

Calcium-transporting systems regulate the ionic concentration of calcium in various compartments according to the different demands of the physiological cycle and these systems upregulate calcium entry by the action of several hormones and calcium binding proteins. Opening of calcium influx channels increases the cytosolic calcium concentrations but high calcium concentrations are toxic to the cell. Because of this toxicity, calcium is rapidly removed from the cytosol by calcium pumps and exchangers.

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Applications

Calcium (Ca^{2+}) is an intracellular second messenger involved in many processes controlling cell homeostasis. Ca^{2+} has been identified to participate in many different signaling pathways, being a key player in inter-organelle communication. Perturbations in Ca^{2+} signaling lead to pathophysiological conditions, such as cardiovascular diseases, metabolic, disorders, cancer and neurodegeneration, among others. Additionally, cellular calcium is known to play an important role in apoptosis and the accumulation of calcium can induce various apoptotic pathways in the cell. Ca^{2+} detection can offer a valuable readout for a wide variety of cell models in basic and translational research.

Properties

State	Solid-phase film with embedded SPAchips
Solubility	Soluble in assay buffer (aqueous)
SPAchip® quantity	2.5 million SPAchips
Storage	Store at 2-8 ºC and protected from the light.
Shipping	Room temperature.
Fluorescence	λex 488 nm; λem 520 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™® Calcium 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	$2.5*10^6$ 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
Cell culture media (phenol red free is recommended)

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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:
Calcium flux positive and negative controls (for example, BAPTA-AM and A23187…)

Handling and storage

Precautions for safe handling

Work under sterile conditions. Protect the tubes from light, specially 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 488 nm and detecting fluorescence emission at 520 nm. 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.

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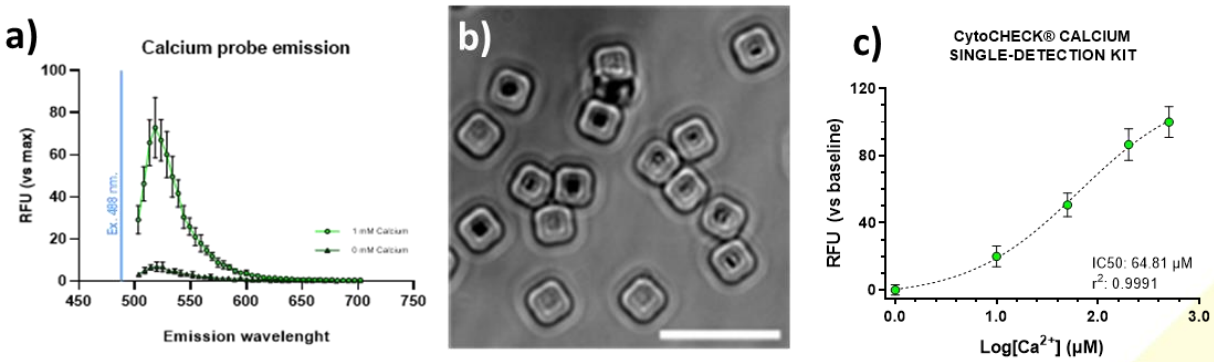

Figure 1: a) Emission spectra of Calcium--sensing probes when excited at the indicated wavelength. b) Brightfield image of SPAchips® once in solution (Image from Torras et al. 2015 DOI: 10.1002/adma.201504164), Scale bar = 10 µm. c) Calibration Curve of Fluorescence Intensity values from CytoCHECK SPAchip® Calcium Single Detection kit at different concentrations.

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 offer a semi-quantitative approach.

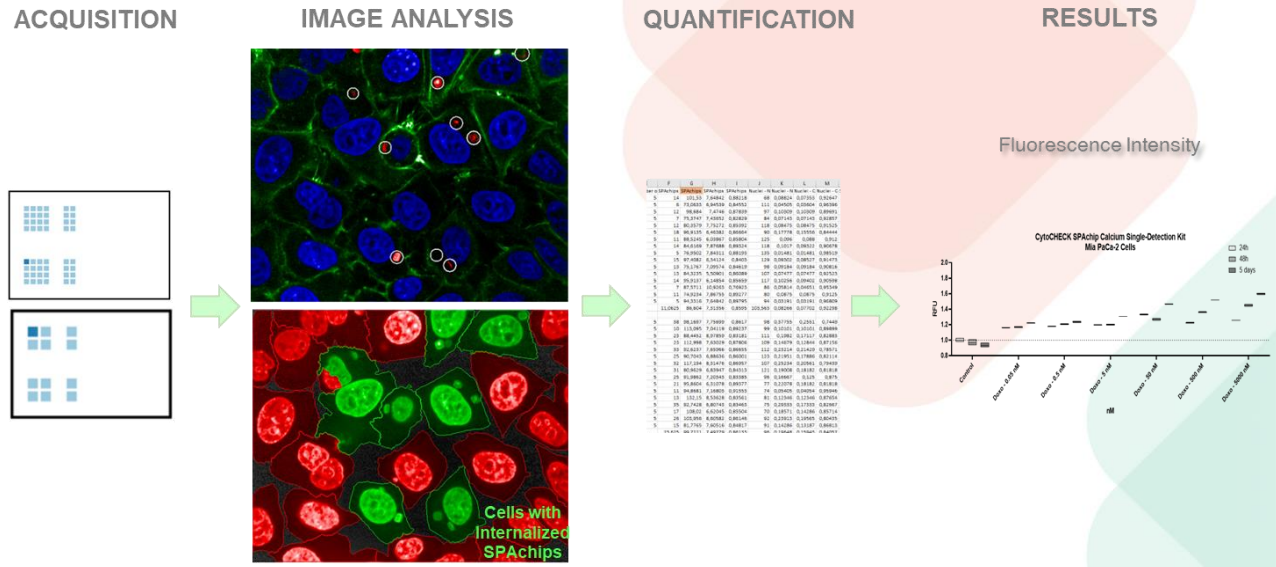

Figure 2: Image Analysis Workflow. From sample data acquisition to object identification, quantification and result presentation.

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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 µm3) 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 sold based on number of SPAchips. 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 amount 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.

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