

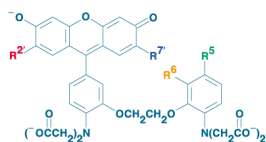
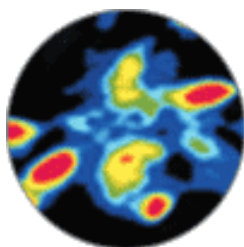
Intracellular Calcium Level

Background and Objectives

The in vitro measurement of intracellular calcium is an essential tool for pharmacological high-content and high throughput screening addressing cell signal transduction events that finally modulate various cellular processes such as gene expression, mitogenesis, metabolism, motility and toxicity. Calcium can enter the cytoplasm of cells either from the extracellular space or by release from intracellular stores. Calcium entry is most commonly induced by ligand-gated and G-protein coupled membrane channels (GPCR) whereas intracellular release is generally triggered by the generation of other signal molecules subsequent to interaction of the cell with different stimuli. Sublethal damage by toxic compounds is often accompanied by changes in intracellular Calcium levels. Monitoring calcium fluxes is a fast and sensitive tool to advance cell-based drug discovery processes.

Experimental Design

The fluorescent indicator "FLUO-4" or "FLUO-4 DIRECT" used in our assays provides improved detection of intracellular Ca^{2+} dynamics. This cell permanent dye can simply be loaded into cells and becomes fluorescent in presence of free calcium inside the cell. Monitoring is made either with flow cytometry or fluorescence spectroscopy. The assay can be multiplexed with other assays.

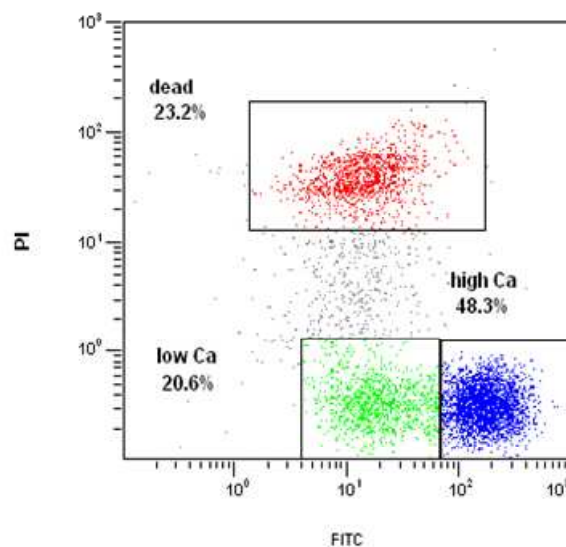


Indicator	$K_d(Ca^{2+})$	R ²	R ⁷	R ⁵	R ⁶
Fluo-3	0.39 μ M	Cl	Cl	CH ₃	H
Fluo-4	0.35 μ M	F	F	CH ₃	H
Fluo-5F	2.3 μ M	F	F	F	H
Fluo-SN	90 μ M	F	F	NO ₂	H
Fluo-4FF	9.7 μ M	F	F	F	F

Calcium Indicators (Invitrogen)

Data Acquisition and Analysis

Your requested cell model will be exposed to the test item and loaded with the calcium indicator to detect intracellular calcium levels in comparison to a untreated control and counterstained with propidium iodide. A time kinetic or dose kinetic is the output of the experiment and analysis will generally be conducted via flow cytometry. The generated data will be evaluated as % change in calcium compared to the untreated control and mean fluorescence intensity (MFI) values.



Detection of intracellular calcium via FLUO-4

Supplemental Testing

Further cellular parameters such as membrane integrity, metabolic activity, reactive oxygen species, cell cycle distribution, and genotoxicity can be added. Moreover a multiplexed format measuring different endpoints at once in a 96-well plate is possible.

Please don't hesitate to contact us for a customized quotation

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