Measurement of intracellular Ca2+

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Abstract

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Protocol

Step 1.

Step 2.
The intracellular Ca2+ concentration was estimated by co-incubating the cells with a cell-permeant Ca2 + fluorophore, Rhod-2 AM (2 μM).

Step 3.
Cells were seeded at a density of 102 cells onto a confocal culture dish. Washed with cold PBS and incubated in a 5% CO2 humidified incubator at 37℃ for 20 min after adding 20 μl of Rhod-2 AM working solution.

Step 4.
Next, the cells were washed twice with PBS and the changes of intracellular calcium were evaluated by a laser scanning confocal microscope (Zeiss LSM 710, Germany).

Step 5.
The Rhod-2 AM fluorescence was observed at 525 nm excitation (Ex)/590 nm emission (Em).