NOV 11, 2017

Measurement of intracellular Ca²⁺

PLOS Neglected Tropical Diseases

Mengchen Shi¹

¹Sun Yat-Sen University

Mengchen Shi

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.kcgcstw

External link:

<https://doi.org/10.1371/journal.pntd.0006074>

Protocol Citation: Mengchen Shi 2017. Measurement of intracellular Ca²⁺.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.kcgcstw>

MANUSCRIPT CITATION:

Shi M, Zhou L, Zhao L, Shang M, He T, Tang Z, Sun H, Ren P, Lin Z, Chen T, Yu J, Xu J, Yu X, Huang Y (2017) Csseverin inhibits apoptosis through mitochondria-mediated pathways triggered by Ca²⁺ dyshomeostasis in hepatocarcinoma PLC cells. PLoS Negl Trop Dis 11(11): e0006074. doi: [10.1371/journal.pntd.0006074](https://doi.org/10.1371/journal.pntd.0006074)

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Protocol status: Working

Created: Oct 18, 2017

Last Modified: Mar 01, 2018

PROTOCOL integer ID:
8296

- 1** The intracellular Ca^{2+} concentration was estimated by co-incubating the cells with a cell-permeant Ca^{2+} fluorophore, Rhod-2 AM (2 μM).
- 2** Cells were seeded at a density of 102 cells onto a confocal culture dish. Washed with cold PBS and incubated in a 5% CO_2 humidified incubator at 37°C for 20 min after adding 20 μl of Rhod-2 AM working solution.
- 3** 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).
- 4** The Rhod-2 AM fluorescence was observed at 525 nm excitation (Ex)/590 nm emission (Em).