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intracellular calcium assay

PLOS One

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1 Works for me dx.doi.org/10.17504/protocols.io.baihicb6

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ABSTRACT

This protocol was used for the experiment conducted in " Sodium-calcium exchanger 1 is the key molecule for urinary potassium excretion against acute hyperkalemia"

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0235360

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Shoda W, Nomura N, Ando F, Tagashira H, Iwamoto T, Ohta A, Isobe K, Mori T, Susa K, Sohara E, Rai T, Uchida S (2020) Sodium–calcium exchanger 1 is the key molecule for urinary potassium excretion against acute hyperkalemia. PLoS ONE 15(6): e0235360. doi: 10.1371/journal.pone.0235360

DOI

dx.doi.org/10.17504/protocols.io.baihicb6

PROTOCOL CITATION

Wakana Shoda, Naohiro Nomura, Fumiaki Ando, Hideaki Tagashira, Takahiro Iwamoto, Akihito Ohta, Kiyosih Isobe, Takayasu Mori, Koichiro Susa, Eisei Sohara, Tatemitsu Rai, Shinichi Uchida 2020. intracellular calcium assay . **protocols.io**
dx.doi.org/10.17504/protocols.io.baihicb6

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Shoda W, Nomura N, Ando F, Tagashira H, Iwamoto T, Ohta A, Isobe K, Mori T, Susa K, Sohara E, Rai T, Uchida S (2020) Sodium–calcium exchanger 1 is the key molecule for urinary potassium excretion against acute hyperkalemia. PLoS ONE 15(6): e0235360. doi: 10.1371/journal.pone.0235360

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0235360

CREATED

Dec 16, 2019

LAST MODIFIED

Jul 01, 2020

Citation: Wakana Shoda, Naohiro Nomura, Fumiaki Ando, Hideaki Tagashira, Takahiro Iwamoto, Akihito Ohta, Kiyosih Isobe, Takayasu Mori, Koichiro Susa, Eisei Sohara, Tatemitsu Rai, Shinichi Uchida (07/01/2020). intracellular calcium assay . https://dx.doi.org/10.17504/protocols.io.baihicb6

PROTOCOL INTEGER ID

31017

MATERIALS TEXT

Calcium Kit - Fluo 4 (Dojindo, Kumamoto, Japan)

- Fluo 4-AM ..... 50 µg × 10
- Dimethylsulfoxide .....2 ml × 1
- Recording Medium (2X) ..... 100 ml × 1
-Probenecid ..... 1.3 ml × 1
-5% Pluronic® F-127 ..... 2.5 ml × 1

35mm glass bottom dish (Iwaki, Shizuoka, Japan)

1 Creation of Loading Buffer: solution A+ solution B
■Solution A
5ml Recording Buffer
50µl Probenesid
80µl Pluronic F-127
approximate 5 ml pure water (up to 10ml)
■Solution B
50 µl DMSO
50µ Fluo4

Loading Buffer should be heated at 37 ℃

2 Cultured cells in glass bottom dish should be washed with PBS, and replaced by 1.2 ml loading buffer.
Then, Incubate at 37 °C for 1 hour. The drugs are added during this period.

3 Preparation for Recording Buffer while cells are incubated in loading buffer.
■Recording Buffer
5ml Recording Medium
50 µl Probenesid
approximate 5ml pure water (up to 10ml)

4 After 1 hour incubation of loading buffer, the cells are washed with PBS , then replaced by 1.2 ml of recording buffer.

5 Following the administration of stimuli (ex. 10mM KCl), the fluorescence intensities of Fluo-4 are quantified from five regions of interest using LSM 510 Meta confocal microscopy and the ZEN 2009 software (Carl Zeiss, Oberkochen, Germany).

Citation: Wakana Shoda, Naohiro Nomura, Fumiaki Ando, Hideaki Tagashira, Takahiro Iwamoto, Akihito Ohta, Kiyosih Isobe, Takayasu Mori, Koichiro Susa, Eisei Sohara, Tatemitsu Rai, Shinichi Uchida (07/01/2020). intracellular calcium assay . https://dx.doi.org/10.17504/protocols.io.baihicb6