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intracellular calcium assay

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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"

MATERIALS

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)

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- 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
 - 50µl Fluo4

Loading Buffer should be heated at 37 °C
- 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).