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EZCell™ Calcium Detection Kit (Cell-Based)

4/18

(Catalog # K432-50; 50 assays; Store at -20°C)

I. Introduction:

Calcium is essential for all living organisms. Calcium sequestration and release into the cytoplasm function as a signal for many cellular processes. As an important second messenger, calcium is involved in many physiological processes such as nerve impulse transmission, muscle contraction, etc. Intracellular calcium is mainly stored in mitochondria and endoplasmic reticulum, and its concentration in cytosol is tightly regulated. Dysfunction of calcium homeostasis causes oxidative stress, cell death and pathogenesis of Alzheimer's disease. BioVision has developed an EZCell™ Calcium Staining Kit that contains membrane permeable, intracellular calcium probe. Upon cell entry, the calcium probe is hydrolyzed by intracellular esterases rendering it membrane impermeable. The cleaved calcium probe binds to intracellular calcium generating fluorescence. The intensity of fluorescence is directly proportional to intracellular calcium levels. A Positive Control reagent is also provided, and serves as experimental control, which elevates intracellular Calcium levels resulting in increased calcium staining. This easy-to-use, non-radioactive kit allows studying the regulation of calcium at the cellular level by using Fluorescence Microscopy and Flow Cytometry in cultured cells.

II. Applications:

- Staining for Intracellular Calcium.
- Screening for compounds that affect calcium signaling.

III. Sample Type:

- Suspension or adherent cells cultures

IV. Kit Contents:

Components	K432-50	Cap Code	Part Number
Assay Buffer (50X)	2 X 1.8 ml	Brown	K432-50-1
Calcium Probe	1 vial	Clear	K432-50-2
Experimental Control (1000X)	40 µl	Yellow	K432-50-3

V. User Supplied Reagents and Equipment:

- Tissue culture plates, 1X PBS, Fetal Bovine Serum and cell culture media
- Fluorescence microscope
- Flow cytometer with excitation filter at 488 nm wavelength (FL1)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Assay Buffer (50X):** Dilute each vial (50X) into 88.2 ml of **sterile** 1X PBS, mix well and keep on ice while in use. Keep at 4°C.
- **Calcium Probe:** In a tissue culture hood prepare a 250X stock solution by adding 78 µl of dry DMSO to Calcium Detector vial, mix Store at -20°C, avoid repeated freeze/thaw cycles, use within two months.
- **Experimental Control (1000X):** Warm to room temperature before use. Aliquot and store at -20°C, avoid repeated freeze/thaw cycles, use within two months.

VII. EZCell™ Intracellular Calcium Detection Protocol:

This protocol was developed using Jurkat cells and HeLa cells cultivated in 6-well tissue culture plates and can be adjusted for any cell type. The assay volume is 1 ml and it should be adjusted accordingly for different plate formats. Optimal conditions depend on the cell type and test compound, therefore we suggest an initial test of several concentrations of Calcium Probe and Positive Control reagent to find the best conditions for your experimental design.

1. Sample Preparation:

a. Obtain suspension or adherent cell culture of desired density and incubate the cells for 8-12 hours in appropriate medium supplemented with 10% FBS at 37°C with 5% CO_2.

b. **For adherent and suspension cells:** Include appropriate controls: **Negative control** – cells not exposed to the Calcium Probe or treatment, **Positive control** –cells to be incubated with 1X Calcium Probe only, **Experimental control** – cells incubated in presence of 1X Experimental Control reagent. Remove the media and replace it with 1 ml of culture media with 0.5% FBS containing either vehicle, 1X Experimental Control reagent or test compounds at desired concentration. Incubate the cells for additional 30 minutes at 37°C with 5% CO_2, or time required by your experimental protocol. Pellet the suspension cells at 400 x *g* for 5 minutes at 4°C temperature and remove media; *use these settings throughout the entire protocol.*

c. Terminate the experiment. Trypsin can be used to collect adherent cells. Centrifuge detached adherent and/or suspension cells at 400 x *g* for 5 minutes at 4°C. Wash the cell pellets twice in 500 µl of ice-cold 1X Assay Buffer. Centrifuge the cells between washes as above and discard the supernatant.

d. Re-suspend cell pellets in 250 µl of medium with 0.5% FBS and add 1 µl of 250X Calcium Probe to Test, vehicle, Positive and Experimental Control cells. Incubate 45 min at 37°C and 5% CO_2. Remove the supernatant after centrifuging cells at 400 x *g* for 5 min at 4°C.

e. Re-suspend cell pellets in 1 ml of Assay Buffer and incubate for 2 minutes at RT. Cells are ready for analysis for calcium staining by flow cytometer and fluorescence microscope.

2. FACS and Fluorescence microscope analysis:

a. FACS acquisition: select the main cell population in the FSC vs. SSC plot to exclude dead cells and cellular debris from Negative Control Cells. Within the main cell population, mean fluorescence intensity in FL1 (Calcium Staining) can be quantified and compared between untreated cells and cells treated with test compounds or between different cell types.

b. Fluorescence Microscope analysis: Observe the cells under the fluorescence microscope for green (Calcium staining) and Blue (Nuclear Staining) fluorescence respectively.

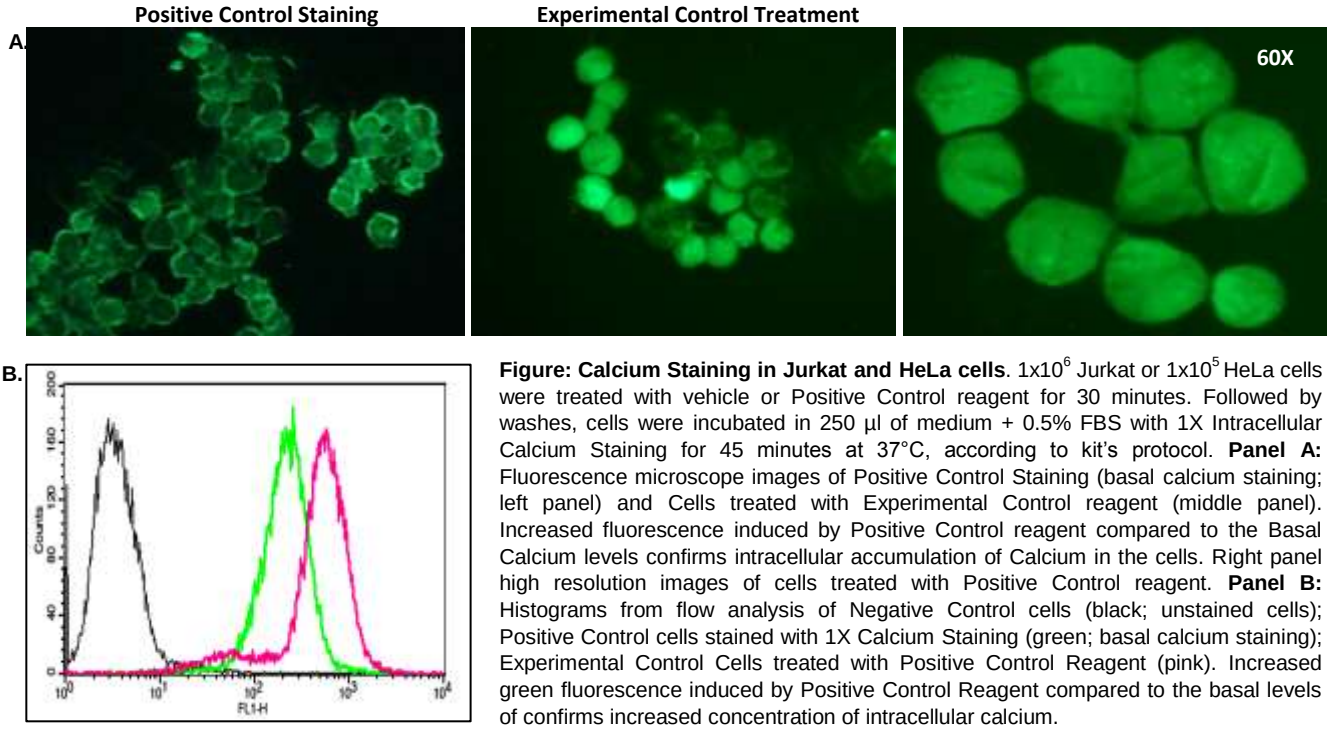

Figure: Calcium Staining in Jurkat and HeLa cells. 1x10^6 Jurkat or 1x10^5 HeLa cells were treated with vehicle or Positive Control reagent for 30 minutes. Followed by washes, cells were incubated in 250 µl of medium + 0.5% FBS with 1X Intracellular Calcium Staining for 45 minutes at 37°C, according to kit's protocol. **Panel A:** Fluorescence microscope images of Positive Control Staining (basal calcium staining; left panel) and Cells treated with Experimental Control reagent (middle panel). Increased fluorescence induced by Positive Control reagent compared to the Basal Calcium levels confirms intracellular accumulation of Calcium in the cells. Right panel high resolution images of cells treated with Positive Control reagent. **Panel B:** Histograms from flow analysis of Negative Control cells (black; unstained cells); Positive Control cells stained with 1X Calcium Staining (green; basal calcium staining); Experimental Control Cells treated with Positive Control Reagent (pink). Increased green fluorescence induced by Positive Control Reagent compared to the basal levels of confirms increased concentration of intracellular calcium.

VIII. RELATED PRODUCTS:

EZCell™ Intracellular Zinc Staining Kit (K525)
GluTracker™ Glucose Uptake Assay Kit (K681)
2-NBDG Glucose Uptake Assay Kit (K682)
GluTracker™ Glucose Uptake Assay Kit (Cell-Based) (K681)
Self-Quenched BODIPY FL Conjugate of BSA (Green) (7632)
EZCell™ Phagocytosis Assay Kit (Red Zymosan) (K398)
Lysosomal Intracellular Activity Assay (Cell-Based) (K448)
EZCell™ Direct Glucose Uptake Assay Kit (K924)
1,5-Anhydroglucitol Uptake Assay Kit (K684)
Propidium Iodide (1056)
EZCell™ Phagocytosis Assay Kit (Red E. coli) (K964)
EZCell™Glutathione Detection Kit (Blue Fluorescence) (K504)

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