Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

EZCell™ Intracellular Zinc Detection Kit (Cell-Based)

11/18

(Catalog # K525-50; 50 assays; Store at -20°C)

I. Introduction:

Zinc (Zn, Atomic Number: 30) is an essential mineral of great biological significance. It is a metal with characteristics similar to Magnesium due to its size and +2 oxidation state. It is the second most abundant transition metal in living organisms after iron. Zn^{2+} binding proteins account for nearly 50% of the transcription regulatory proteins in the human genome and require zinc as an essential cofactor in the regulation of gene expression. Numerous enzymes also employ Zn^{2+} ions in their active sites. Free Zn^{2+} is released from metalloprotein complexes during oxidative stress with the remainder being bound to proteins or nucleic acids. Other examples of biological roles of Zinc include signal transduction, regulation of apoptosis, neurotransmission, synaptic plasticity and prostate gland function. In humans, Zinc deficiency is associated with many chronic diseases such as renal disease, sickle cell anemia, cirrhosis, some cancers and deficiencies in metabolism. BioVision has developed the EZCell™ Intracellular Zinc Staining Kit (cell-based) which contains a selective and membrane permeable fluorescent probe for detection of intracellular Zinc. We also include a Positive Control that elevates Zinc staining, and cell-permeable Zinc Chelator that reduces fluorescence induced by Positive Control. This easy-to-use non-radioactive assay allows studies of the regulation of Zinc at the cellular level by Fluorescence Microscopy and Flow Cytometry in cultured cells.

II. Applications:

- Staining for intracellular Zinc.
- Mechanistic study for intracellular Zinc homeostasis.

III. Sample Type:

- Suspension or adherent cells cultures

IV. Kit Contents:

Components	K525-50	Cap Code	Part Number
Assay Buffer (10X)	25 ml	NM	K525-50-1
Zinc Probe (500X)	0.1 ml	Orange	K525-50-2
Positive Control (2000X)	100 µl	Yellow	K525-50-3
Zinc Chelator (500X)	100 µl	Purple	K525-50-4

V. User Supplied Reagents and Equipment:

- Phosphate Buffered Saline (PBS, pH 7.4)
- Tissue culture plates and media
- Fluorescence microscope
- Flow cytometer with filter at 488 nm wavelength and at 530 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 entire protocol before performing the assay.

- **Assay Buffer (10X):** Dilute 10X Assay Buffer 10 times in ddH2O to obtain a 1X Assay Buffer. Keep on ice while in use.
- **Zinc Probe (500X), Positive Control (2000X) and Zinc Chelator (500X):** Warm to room temperature before use. Store at -20°C, avoid repeated freeze/thaw cycles.

VII. EZCell™ Intracellular Zinc Staining Protocol:

This protocol was developed for Jurkat cells and can be adjusted for any cell type. The assay volume is 1 ml; however the number of cells and assay volume should be adjusted accordingly for different plate formats. Optimal conditions depend on the cell type, therefore we recommend assay optimization.

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. Next day, remove the media from adherent cells and replace it with 1 ml of 1 X Assay Buffer containing either vehicle or the test compound at desired concentration. For suspension cells: pellet the cell culture at 300 x g for 5 minutes at room temperature prior to media removal. Re-suspend the cells in 1 X Assay Buffer enough to accommodate proper controls and test compounds. ***Use this centrifugation setting for suspension cells throughout the entire protocol prior removal of media and washes*.**

c. To prepare proper controls, add the appropriate kit components as follows: **Negative Control:** Unstained and untreated cells; **Positive Control:** Dilute the Positive Control (2000X) 10 fold by adding 10 µl of Positive Control (2000X) into 90 µl dH_2O, mix well. Add 5 µl of Positive Control (200X) per 1 ml of cell suspension. **Experimental Control:** add 5 µl of Positive Control (200X) and 2 µl of Zinc Chelator (500X) per 1 ml of cell suspension. Incubate the cells for 30 minutes at 37°C with 5% CO_2, or time required by your experimental protocol.

d. **Zinc Staining:** For adherent and suspension cells: Terminate the experiment and harvest the cells. Wash the cells twice in 1 ml of ice-cold 1X Assay Buffer. Re-suspend the cell pellets in 1 ml of 1X Assay Buffer. Add 2 µl of Zinc Probe (500X) into 1 ml of cell suspension; incubate for 30 min at 37°C, 5% CO_2. Remove the stain and wash cells twice with 1X Assay Buffer. Re-suspend the cells in 1 ml of 1X Assay Buffer. Cells are ready to be analyzed by flow cytometer or fluorescence microscope for Zinc staining. **Note:** Trypsin can be used to collect the adherent cells prior to FACS analysis.

e. **FACS acquisition and analysis**: 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 Ex/Em = 488/530) (Zinc Staining) can be quantified and compared between untreated cells and cells treated with test compounds, or between different cell types.

f. **Fluorescence microscope analysis:** Observe the cells under the fluorescence microscope for Green (Zinc staining) and Blue (Nuclei staining) fluorescence respectively.

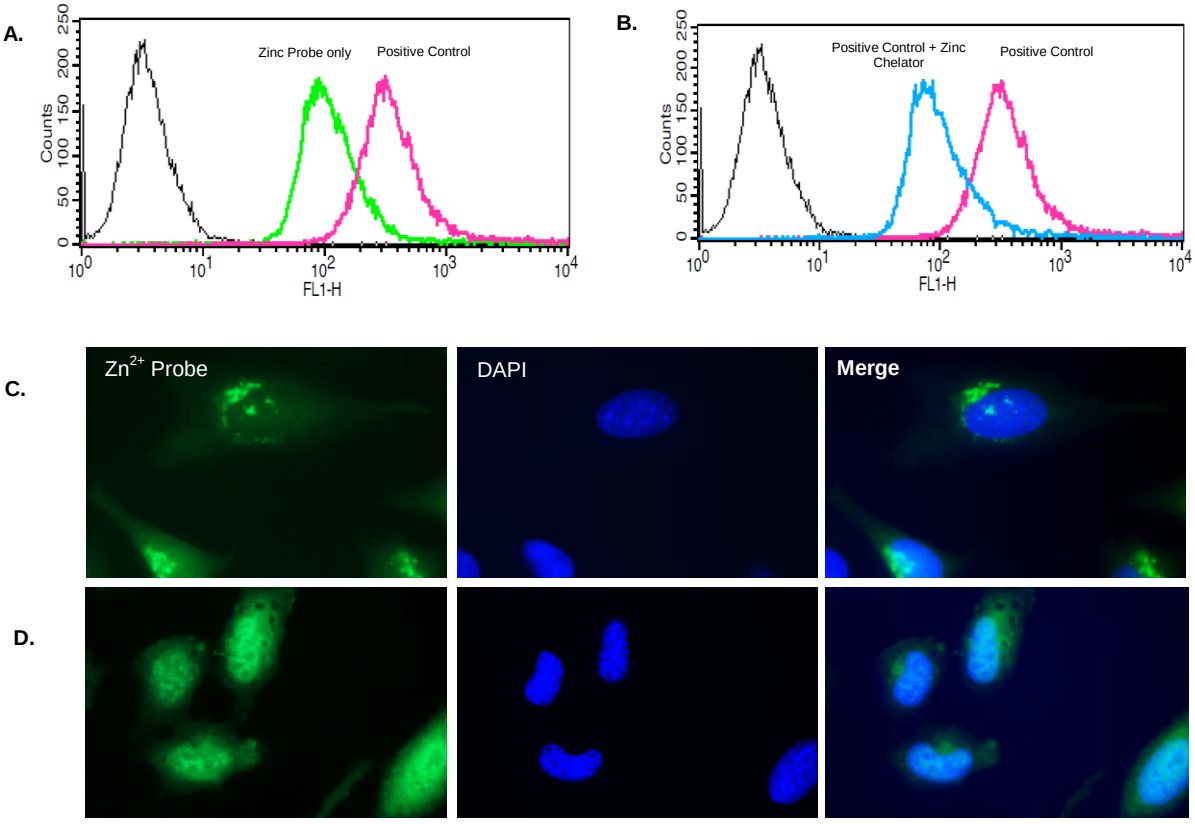

Figure: Zinc Staining in Jurkat and HeLa cells. 1x10^6 Jurkat or 1x10^5 HeLa cells were treated with vehicle or Positive Control, or Positive Control plus Zinc Chelator for 30 minutes followed by Zinc Staining according to kit's protocol. **A.** Histograms from flow analysis of untreated cells stained with Zinc Probe (Green Line) and cells treated with Positive Control (Pink Line) show increased signal in cells treated with Positive control reagent compared to untreated cells. **B.** Positive Control Treated Cells without (Pink Line) and with Zinc Chelator treated Cells (Blue Line). Decreased fluorescence in presence of Zinc Chelator confirms that the fluorescence is generated by intracellular Zinc. **C.** Fluorescence microscope images of Basal Zinc Staining **(Green)** and Positive Reagent treated Cells **(D)**. Increased Green fluorescence induced by treatment with Positive Control compared to the Basal Zinc levels confirms intracellular accumulation of fluorescent stain in the cells.

VIII. RELATED PRODUCTS:

Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucose and Sucrose Colorimetric/Fluorometric Assay Kit (K616)
PicoProbeTM Glucose-6-Phosphate Fluorometric Assay Kit (K687)
Glucose Dehydrogenase Activity Colorimetric Assay Kit (K786)
Glucose-1-Phosphate (G1P) Colorimetric Assay Kit (K697)
GluTrackerTM Glucose Uptake Assay Kit (Cell-Based) (K681)
Glucose AGE-BSA-II (2223)
GPI, human recombinant (7822)
Self-Quenched BODIPY FL Conjugate of BSA (Green) (7632)
EZCell™ Phagocytosis Assay Kit (Green Zymosan) (K397)
EZCell™ Phagocytosis Assay Kit (Red Zymosan) (K398)
EZCell™ Glutathione Detection Kit (Blue Fluorescence) (K504)
Lysosomal Intracellular Activity Assay (Cell-Based) (K448)
Lysosomal Cytotoxicity Kit
Glucose Uptake Fluorometric Assay Kit (K666)
Glucose Oxidase Activity Colorimetric/Fluorometric Assay Kit (K788)
Glucose-6-Phosphate Colorimetric Assay Kit (K657)
Chloroquine Diphosphate (1825)
Propidium Iodide (1056)
PicoProbeTM Glucose Fluorometric Assay Kit (K688)
Maltose and Glucose Colorimetric/Fluorometric Assay Kit (K618)
EZCell™ Phagocytosis Assay Kit (Green E. coli) (K963)
EZCell™ Phagocytosis Assay Kit (Red E. coli) (K964)
GLUT4 Antibody (3945)

FOR RESEARCH USE ONLY! Not to be used on humans.