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

EZCell™ Thiol Detection Kit

10/20

(Catalog# K2063-100; 100 assays; Store at -20 °C)

I. Introduction:

Thiols are organosulfur compounds that contain a free sulfhydryl group (-SH). The most predominant intracellular thiols in eukaryotes are cysteine, glutathione and cysteine-containing proteins. Thiols are vital in mediating cellular redox chemistry, preventing oxidative damage by reactive oxygen species and assisting in proper protein folding via the formation of disulfide bridges. Under physiological conditions, glutathione (GSH) is the most abundant intracellular thiol. High levels of reduced glutathione in the cytosol help maintain a reduced redox state, as GSH can react with the electrophilic xenobiotics and reduce protein thiols to support a variety of enzymatic redox reactions. Depletion of intracellular GSH and other thiols results in the buildup of lipid peroxidation products, indiscriminate oxidation of protein cysteine residues and is a strong activator of apoptosis. **BioVision's EZCell™ Thiol Detection Kit** utilizes a cell-permeable, thiol reactive fluorogenic Thiol Dye. The dye is strongly quenched and is essentially non-fluorescent until it reacts with an intracellular thiol moiety to generate a bright green fluorescence. The fluorescence can be quantified by measuring the fluorescence intensity in the FL1 channel (Ex/Em = 492/517 nm) of a flow cytometer. The thiol dye is selective for free reduced thiols, reacting with reduced GSH and cysteine to form highly fluorescent adducts, and does not label oxidized GSSG. The fluorescent conjugates are stable and are directly proportional to the intracellular thiol level. The EZCell™ Thiol Detection Kit provides a rapid, non-radioactive and sensitive method for measuring the overall intracellular thiol level in living cells and for studying the effects of oxidative stress and other thiol-modifying conditions.

II. Applications:

- Detection of intracellular thiol level.
- Assessment of drugs, which affect intracellular thiol level.

III. Sample Type:

- Adherent or Suspension cells

IV. Kit Contents:

Components	K2063-100	Cap Code	Part Number
Thiol Assay Buffer	100 ml	NM	K2063-100-1
Thiol Dye	1 vial	Red	K2063-100-2
DMSO	1 ml	Amber	K2063-100-3
Thiol Inducer	1 vial	Green	K2063-100-4

V. User Supplied Reagents and Equipment:

- Tissue Culture treated 6-well plate with clear bottom and lid
- Sterile PBS
- Trypsin / 0.25% EDTA
- Cell Culture Medium and Fetal Bovine Serum (FBS)
- Flow Cytometer

VI. Reagent Preparation and Storage:

Store the kit at -20 °C, protected from light. Warm all reagents to room temperature (RT) before use. Read the entire protocol before performing the assay.

- **Thiol Assay Buffer:** Warm to 37 °C before use. Store at 4 °C under sterile conditions.
- **Thiol Dye:** Add 45 µl of DMSO to the vial to make 250X Thiol Dye. Mix well and protect it from light. Store at -20 °C for 2 months.
- **DMSO:** Warm at RT to thaw completely.
- **Thiol Inducer:** Reconstitute the vial in 214 µl DMSO to prepare 1000X Thiol Inducer. Mix well and store at -20 °C for 2 months.

VII. Thiol Detection Protocol:

This assay was developed with HeLa (adherent) and Jurkat (suspension) cells, but can be modified for any cell lines. The protocol below is for a 6-well tissue culture plate. Adjust volumes accordingly for other plate formats. The assay volume is 500 µl. Cell density varies with the cell type. It may be necessary to adjust the cell numbers to optimize cell density. Optimize the treatment time with the Thiol Inducer for the cell type to be tested. Culture cells (3 - 5 x 10^5 cells/well) in a 6-well plate (final volume of 2 ml/well culture medium) and incubate for 24 hr at 37 °C in a sterile 5% CO_2 atmosphere incubator. **Note *(Optional)*:** Treat cells in the absence or presence of various amounts of the test compound. Incubate cells in culture medium with 0.1% FBS plus test compound for 1- 24 hr. This step is required to study the potential effects of the test compound on the activation or depletion of intracellular thiol levels.

1. **Preparation of Cells:** Grow cells (adherent or suspension) in appropriate media at 37 °C in a 5% CO_2 cell culture incubator overnight to obtain at least 1 x 10^6 cells per assay conditions. Set up cells into 4 groups as follows: **Negative Control:** Cells that are not exposed to treatment or thiol dye; **Background Control:** Cells that are incubated with the thiol dye; **Positive Control:** Cells that are treated with thiol inducer and incubated with thiol dye and **Experimental Group:** Cells that are treated with drugs of interest.
2. **Preparation of 1X Thiol Inducer Solution:** Add 2 µl of 1000X Thiol Inducer into 1998 µl of cell culture media with 0.1% FBS (pre-warm at 37 °C) and mix well. Discard the diluted Thiol Inducer after use.

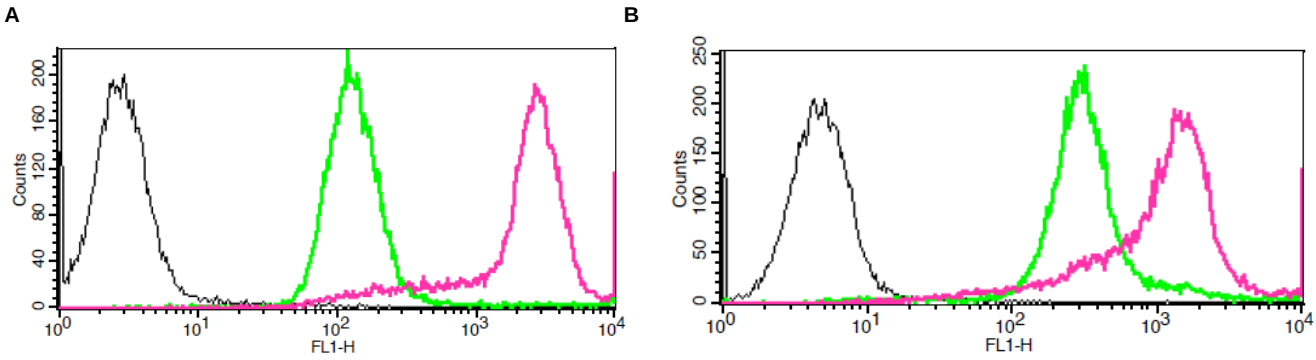
3. For Adherent Cells:

a. Remove the cell media and wash with 1 ml Thiol Assay Buffer.
b. Add 1000 µl of 1X Thiol Inducer Solution to the Positive Control group only. For Negative and Background Controls, add 1000 µl Thiol Assay buffer. For Experimental Group, dilute the drug of interest to 1000 µl with Thiol Assay Buffer. Incubate plate(s) at 37 °C for 2 hr. **Note:** The incubation time needs to be optimized depending on the cell type.
c. Remove the supernatant and wash with 1 ml Thiol Assay Buffer once.
d. Trypsinize and collect cells in 1 ml complete medium with 10% FBS into 1.5 microcentrifuge tubes. Punch a hole in the top of the tube cap by using 18 G needle.
e. Centrifuge the cells at 500 x *g* for 5 min and remove the supernatant
f. Add 1 ml PBS to the cell pellet and mix by pipetting up and down. Centrifuge at 500 x *g* for 5 min and remove the supernatant.
g. Dilute the 250X Thiol Dye 250 fold with Thiol Assay Buffer to make 1X Thiol Dye (e.g. mix 4 µl of 250X Thiol Dye with 996 µl of Thiol Assay Buffer). Add 100 µl of 1X Thiol Dye into Background Control, Positive Control and Experimental Groups and tap with finger to gently mix. Add 100 µl Thiol Assay Buffer in the Negative Control group tube(s) and incubate at 37°C for 30 min.
h. Centrifuge cells at 500 x *g* for 5 min and remove the supernatant.
i. Add 1 ml Thiol Assay Buffer. Mix well and centrifuge the cells at 500 x *g* for 5 min and remove the supernatant
j. Resuspend the cells in 500 µl Thiol Assay Buffer.

4. For Suspension Cells:

a. Collect cells in 1.5 ml microcentrifuge tubes. Centrifuge cells at 500 x *g* for 5 min and remove the supernatant.
b. Wash with 1 ml Thiol Assay Buffer. Centrifuge cells at 500 x *g* for 5 min and remove the supernatant.
c. Add 1000 µl of 1X Thiol Inducer Solution to Positive Control group only. For Negative and Background Controls, add 1000 µl Thiol Assay buffer. For Experimental Group, dilute the drug of interest to 1000 µl with Thiol Assay Buffer. Incubate plate(s) at 37 °C for 2 hr.
Note: The incubation time needs to be optimized depending on the cell type.
d. Centrifuge cells at 500 x g for 5 min and carefully remove the supernatant.
e. Wash cells once with 1 ml of Thiol Assay Buffer. Mix well and centrifuge cells at 500 x *g* for 5 min. Carefully remove the supernatant.
f. Dilute of the 250X Thiol Dye 250 fold with Thiol Assay Buffer to make 1X Thiol Dye (e.g. mix 4 µl of 250x Thiol Dye into 996 µl of Thiol Assay Buffer). Add 100 µl of 1X Thiol Dye into Background Control, Positive Control and Experimental Groups. Tap with finger to gently mix. Add 100 µl Thiol Assay Buffer to the Negative Control group tube(s) and incubate at 37 °C for 30 min.
g. Centrifuge cells at 500 x *g* for 5 min and remove the supernatant.
h. Add 1 ml Thiol Assay Buffer and mix well. Centrifuge the cells at 500 x *g* for 5 min and remove the supernatant
i. Resuspend cells with 500 µl Thiol Assay Buffer.

5. Data Analysis. Analyze by Flow Cytometry in FL-1 channel. Establish forward and side scatter gates using the Negative Control cells to exclude the cell debris and cell aggregates.



Figures: Detection of Thiol level in Jurkat Cells and HeLa Cells: A. Jurkat cells were seeded at 1x10^6 cells/well into a 6-well tissue culture plate with 10% FBS culture media. B. HeLa cells were seeded at 5 x 10^5 cells/well into 6-well tissue culture plate with 10% FBS culture medium for 24 hours. Cells were either not treated **(Negative Control, Black Line)**, or treated with 1XThiol Inducer for 2 hr at 37 °C, 5% CO_2.The intracellular Thiol level were analyzed by flow cytometry according to the kit protocol. Fluorescence intensity was detected and recorded on a BD flow cytometer in FL-1 channel after staining with Thiol Dye: Background Control (Green line); Positive Control (Pink line).

VIII. RELATED PRODUCTS:

EZCell™ Glutathione Detection Kit (K504)
EZCell™ Cell Cycle Analysis Kit (K920)
Brdu Cell Proliferation Assay Kit (K306)
EZCell™ Migration/Chemotaxic Assay Kit (K908)
Reactive Oxygen Species (ROS) Detection Assay Kit (K936)
Annexin V-FITC Apoptosis Detection Kit (K201)
EZCell™ Invasion Assay (K917)
EZCell™ Intracellular Nitric Oxide Synthase (NOS) Kit (K207)
EZCell™ Direct Glucose Uptake Assay Kit (K924)
EZClick™ TUNEL-in situ DNA Fragmentation/Apoptosis Kit (K191)

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