

KLD-004

Intracellular ThioIEZ™ Cell Survival Assay

Total intracellular Thiol Assay Kit (patent pending)

Assay Description

Intracellular ThioIEZ™ is an easy to use assay for measurement of thiols in cells. Glutathione is a natural antioxidant and a major thiol that limits cellular damage in healthy tissues during oxidative stress. It is also centrally involved in repairing damage induced by cancer drugs and radiation and in the detoxification of several commonly used cancer chemotherapeutic drugs. These processes cause altered glutathione homeostasis in both normal and cancer tissues. Glutathione homeostasis by recycling of oxidized glutathione is necessary for the survival of cells. Cysteine is a thiol precursor for Glutathione synthesis and is present in cells with a 10 fold lower concentration when compared with Glutathione levels. **Intracellular ThioIEZ™** measures total intracellular thiols including glutathione and cysteine in a simple and user friendly assay. It may have multiple applications for research in aging, oxidative stress, antioxidant screening, chemotherapy response and toxicology.

Safety Precautions

Eye, skin and respiratory irritants are contained in this kit. Do not ingest or inhale. Utilize standard laboratory safety procedures when handling these reagents.

FOR LIFE SCIENCE RESEARCH USE ONLY.

Chemicals contained in this kit: Dithiobisnitrobenzoic acid, ethylene diamine tetraacetic acid, phosphate buffered saline, sodium phosphate, sulfosalicyclic acid.

Kit Reagents

Reagent 1 (KLD-A004; **white** bottle) – Store at 2 - 25° C

Reagent 2 (KLD-B004; **clear** bottle) – Store at 2 - 8° C

Reagent 3 (KLD-C004; **amber** bottle) – Store at 2 - 8° C

Approximate uses: 100 assays using a 96-well plate.

Intracellular ThioIEZ™ Assay

Step 1

Plate cells at the desired cell density per ml normal growth medium in a 6 well plate the day before the assay.

Step 2

Remove the growth medium. Wash three times with 3ml Earle's Balanced Salt Solution (EBSS).

Step 3

Add 50 μ l of **Reagent 1** to each well of a 6 well plate and gently swirl the plate five times for mixing. Incubate for 10 min on ice.

Step 4

Mix the content of the well with a cell scraper and transfer the content of each well into a microfuge tube. Vortex gently for 20 secs and centrifuge at 9000 rpm for 3 min at 4°C. Store the supernatant at 4°C for short term storage (2 days) or -20°C for long term storage (1-2 weeks). Use the supernatant for total thiol assay as described in Step 5.

Step 4

1. Use 7ml glass tubes for the assay.
2. Prepare the tubes as described in the table.
3. Use the supernatant prepared in Step 3.

Sample	Reagent 2	Reagent 3	Supernatant volume
Blank	1350 μ l	150 μ l	0 μ l
Cell sample	1050 μ l	150 μ l	300 μ l

Vortex gently and leave at RT for 3-5min.

Read the absorbance at 412 nm in a spectrophotometer.

Subtract the blank from the experimental samples.

Notes

This assay gives a linear response for mammalian cells (0; 100,000, 200,000, 400,000, 600,000, 800,000) plated in 1ml growth medium in a six well plate with up to 15% fetal bovine serum and measured 20 hours after plating.