ROCKLAND
antibodies & assays

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KLD-001
CellCountEZ™ Cell Survival Assay
Toxicity, Proliferation, and Survival (TPS) Assay™ Kit (patent pending)

Assay Description

There is great interest in improved methods to quantitate cell proliferation and survival, which are important for applications in cell biology, toxicology, drug screening and many other biosciences. All current approaches on the market in use for several decades have a variety of disadvantages, including non-linearity, high background and cumbersome, costly and time consuming protocols.

CellCountEZ™ is a tissue culture media-based assay that can measure metabolically active live cells and quantify cell death caused by radiation, chemotherapeutics or toxins.

The compound in **CellCountEZ™** is superior to existing compounds used for quantifying cell proliferation and survival because it is readily soluble, membrane permeable and converted by live cells intracellularly before transport into the extracellular culture media. Cell media is used in the assay, avoiding the need to lyse cells and thereby saving time and cost while preserving the ability to perform other cellular tests in the same culture system. Thus, **CellCountEZ™** offers many advantages that make it superior to common existing methods for quantifying cell growth and survival.

HEDS Pathway

CellCountEZ™ is based on the ability of mammalian cells to rapidly and efficiently convert hydroxyethyl disulfide (HEDS) into mercaptoethanol (ME) through a bioreduction mechanism (1-3). Bioconversion of HEDS to ME relies on the activity of the oxidative pentose phosphate cycle (OPPC) (Figure 1). The amount of ME produced from HEDS can be measured in the extracellular culture media since ME produced inside cells is extruded quickly by cells through an active transport mechanism. ME is then easily measured in the extracellular medium without the need for cellular lysis and extraction methods.

The following diagram shows the metabolic conversion of HEDS into ME:

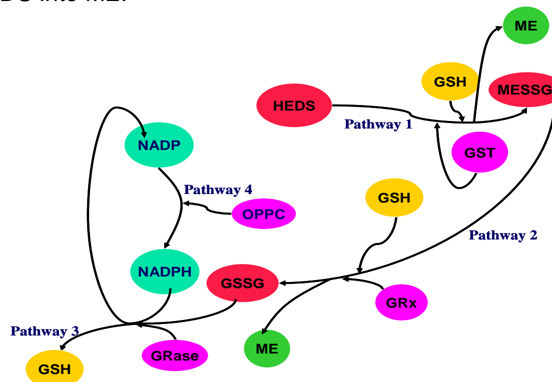

Figure 1: Schematic representation of the various pathways involved in the cellular interactions of HEDS. HEDS reacts spontaneously with glutathione (GSH) or in a reaction catalyzed by glutathione-S-transferase (GST) to produce mixed disulfide (MESSG) of GSH and mercaptoethanol (ME) (Pathway 1). The mixed disulfide MESSG reacts with GSH and produces ME and GSSG by the catalytic action of glutaredoxin (GRX) (Pathway 2). The glutathione disulfide GSSG reacts with NADPH and produces GSH by the catalytic action of glutathione reductase (GRase) (Pathway 3). The conversion of GSSG to GSH i.e. GSH recycling requires NADPH recycling (NADP+ → NADPH) by oxidative pentose phosphate cycle (OPPC) (Pathway 4).

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, hydroxyethyl disulfide, phosphate buffered saline, sodium phosphate.

Kit Reagents

Reagent 1 (KLD-A001; **amber** tube) – Store at 2 - 25° C

Reagent 2 (KLD-B001; **white** tube) – Store at 2 - 8° C

Reagent 3 (KLD-C001; **amber** bottle) – Store at 2 - 8° C

Approximate uses: 1000 assays using a 96-well plate.

Reagent Preparation

Preparation of **Reagent 4**: Transfer 1.2 mL of **Reagent 2** into **Reagent 1** in the amber microfuge tube and vortex for 15-30 seconds. Bring Reagent 4 to room temperature before preparing Reagent 5. [Reagent 4 is stable for up to 6 months if refrigerated in an amber tube.]

Preparation of **Reagent 5**: Five minutes before the assay, add 100 µl of room temperature **Reagent 4** to 900 µl (1:9) of your growth medium with no more than 15% fetal bovine serum in a new microfuge tube and mix it gently with a micropipette. Avoid air bubbles.

TPS Assay™

Step 1
Add 10 µl of **Reagent 5** to each well of a 96-well plate. Incubate for 2 hours at 37° C in a humidified CO_2 incubator.

Step 2
Add 30 µl of **Reagent 3** to each well of the above plate. Incubate for 1-2 minutes at room temperature. Read at 412 nm with a reference wavelength of 650 nm in a microtiter plate reader.

OR

Transfer 80 µl medium from Step 1 into another 96-well plate. Add 30 µl of **Reagent 3** to each well of the above plate. Incubate for 1-2 minutes at room temperature. Read at 412 nm with a reference wavelength of 650 nm in a microtiter plate reader.

Notes

This assay gives a linear response for cells (0; 1,000; 5,000; 10,000; 20,000; 40,000) plated in 100 µl growth medium with up to 15% fetal bovine serum in a 96-well plate and measured 20 hours after plating.

Drug toxicity could be measured for up to 4 days after treatment with drugs using this assay for 5,000 cells plated in 100 µl growth medium with up to 15% fetal bovine serum in a 96-well plate.

References

1. Li J, Zhang D, Ward KM, Prendergast GC, and Ayene IS. Hydroxyethyl disulfide as an efficient metabolic assay for cell viability in vitro. **Toxicol. In Vitro, In Press**.

2. Ayene IS, Biaglow JE, Kachur AV, Stamato TD and Koch CJ: Mutation in G6PD gene leads to loss of cellular control of protein glutathionylation: Mechanism and Implication. **J. Cell Biochem. 103: 123-135, 2008**.
3. Ayene IS, Stamato TD, Mauldin SK, Biaglow JE, Tuttle SW, Jenkins SF and Koch CJ: Mutation in the glucose-6-phosphate dehydrogenase gene leads to inactivation of Ku DNA end binding during oxidative stress. **J. Biol. Chem. 277: 9929-9935, 2002**.