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Protocol

CellCountEZ™ Cell Survival Assay

Toxicity, Proliferation, and Survival (TPS) Assay™ Kit (patent pending)

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.

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 RESEARCH USE ONLY.

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

Reagents Required

Product	Storage	Item No.
Reagent 1	Amber tube, store 2-25°C	KLD-A001
Reagent 2	Clear/White tube, store 2-8°C	KLD-B001
Reagent 3	Amber tube, store 2-8°C	KLD-C001

Reagent Preparation

Preparation of Reagent 4

1. Transfer 1.2mL of Reagent 2 into Reagent 1 into the amber microfuge tube.
2. Vortex for 15-30 seconds.
3. Bring Reagent 4 to room temperature before preparing Reagent 5.
   Note: Reagent 4 is stable for up to 6 months at 2-8°C in an amber tube.

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Preparation of Reagent 5

1. Five minutes before assay, in a new microfuge tube, add (1:9) 100µL of room temperature Reagent 4 to 900µL of your growth medium with no more than 15% fetal bovine serum.
2. Mix gently with a micropipette to avoid air bubbles.

HEDS Pathway

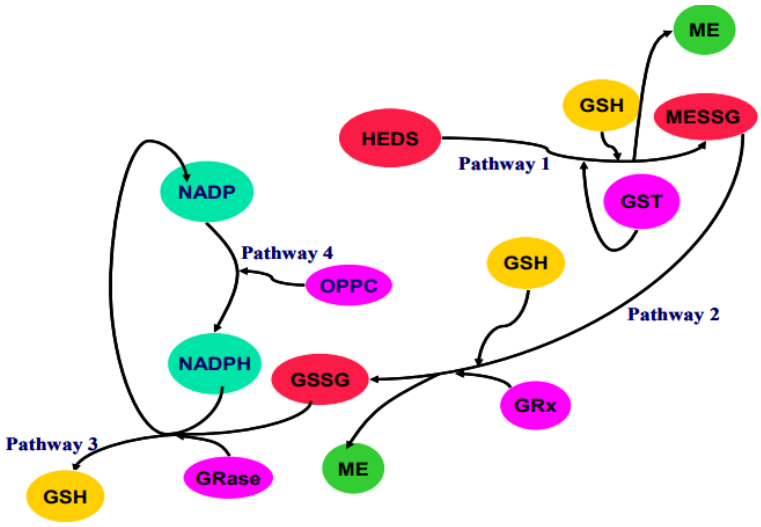

Figure 1: Schematic representation of the various pathways involved in the cellular interactions of HEDS. HEDS reacts spontaneously with glutathione (GSH) or in a reaction catalyzed by glutathione-S-transferase (GST) to produce mixed disulfide (MESSG) of GSH and mercaptoethanol (ME) (Pathway 1). The 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).

TPS Assay™

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.
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.

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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

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