Hydroethidine [Dihydroethidium] *CAS 104821-25-2*

Catalog number: 15200
Unit size: 25 mg

Component	Storage	Amount
Hydroethidine [Dihydroethidium] *CAS 38483-26-0*	Freeze (<-15 °C), Minimize light exposure	25 mg

OVERVIEW

Hydroethidine operates effectively as a probe for measurement of reactive oxygen species. The dye enters cells freely and is dehydrogenated to an ethidium compound. The probe has been used extensively with NK cell and as a vital dye for identification of proliferation and hypoxic cells in tumors. Studies have been performed using neutrophils and endothelial cells as well as HL60 cells and macrophages. A major advantage of this probe is its ability to distinguish between superoxide and H2O2. Fluorescence emission occurs at around 600 nm.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	520 nm
Emission:	600 nm
Cutoff:	550 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Hydroethidine [Dihydroethidium] stock solution:
Make 5-10 mM DMSO stock solution.

Note The unused DMSO stock solution should be aliquoted into single use vial and stored at -20 °C. Keep awayfrom light.

PREPARATION OF WORKING SOLUTION

Hydroethidine [Dihydroethidium] dye working solution:
Make the dye working concentration of 5 – 20 µM in a physiological buffer (such as PBS, HBSS, HEPES).

Note The optimal working concentration for your application must be empirically determined.

SAMPLE EXPERIMENTAL PROTOCOL

1. Add equal volume (such as 100 µL of the cells in growth medium) of the dye working solution to the cells, and incubate the cells at RT or 37°C for 5 to 60 minutes.

2. Determine the baseline fluorescence intensity of a sample of the loaded cells prior to exposing the cells to experimental inducements.

3. Negative controls should be assessed as follows:

   a. Examine the fluorescence of cell-free mixtures of dye and buffer/media with and without the inducer. In the absence of extracellular esterases and other oxidative enzymes, the gradual increase in fluorescence over time may be related to spontaneous hydrolysis, atmospheric oxidation, and/or light-induced oxidation.

   b. Examine the fluorescence of untreated (control) loaded cells that have been maintained in growth medium or buffer. In healthy cells, oxygen radicals are eliminated by cellular enzymes and/or natural antioxidants. Following the dye-loading recovery period, healthy cells should exhibit a low level of fluorescence that is relatively stable for the duration of the experiment; however, a gradual increase (due to auto-oxidation) or decrease (due to loss of dye from cells or photobleaching) in fluorescence may be observed. In the absence of any stimulus or inducement, a burst of fluorescence in healthy, untreated cells could indicate progress to cell death or some other oxidative event.

4. Positive controls may be stimulated with tert-butyl hydroperoxide (TBHP) to a final concentration of ~100 µM (increase or decrease dose based on the sensitivity and response of the cells).

EXAMPLE DATA ANALYSIS AND FIGURES

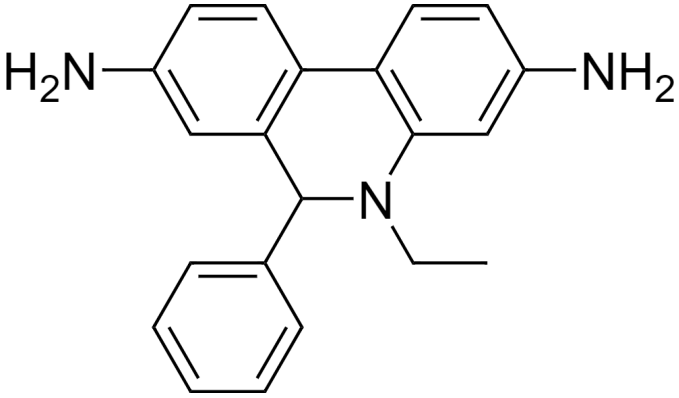

Figure 1. Chemical structure for Hydroethidine [Dihydroethidium] *CAS 104821-25-2*

DISCLAIMER