Hydroethidine [Dihydroethidium]

Ordering Information:	*Storage Conditions:*
Product Number: 15200 (25 mg), 15202 (5 X 1mg)	Keep at -20 °C and desiccated

Chemical and Physical Properties

Molecular Weight: 315.41
Solvent: DMSO
Spectral Properties: Excitation = 518 nm; Fluorescence = 605 nm

Biological Applications

Hydroethidine operates effectively as a probe for measurement of reactive oxygen species. The dye enters cells freely and is dehydrogenated to ethidium bromide. 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.

Sample Protocol for Staining Cells

The following procedures provide a general guideline and should be modified for your particular application.

1. Make 5-10 mM DMSO stock solution. The unused DMSO stock solution should be aliquoted into a single use vial and stored at -20 °C. Keep from light.
2. Make the dye working concentration of 5–20 μM in a physiological buffer (such as PBS, HBSS, HEPES). The optimal working concentration for your application must be empirically determined.
3. Add equal volume (such as 100 μL of the cells in growth medium) of the dye working solution (from Step 2) to the cells, and incubate the cells at RT or 37 ^{o}C for 5 to 60 minutes.
4. Determine the baseline fluorescence intensity of a sample of the loaded cells prior to exposing the cells to experimental inducements.
5. **N**egative controls should be assessed as follows:

   5.1 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.

   5.2 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.
6. 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).

References

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2. Burnaugh L, Sabeur K, Ball BA. (2006) Generation of superoxide anion by equine spermatozoa as detected by dihydroethidium. Theriogenology.
3. Fernandes DC, Wosniak J, Pescatore LA, Bertoline MA, Liberman M, Laurindo F, Santos CX. (2006) Analysis of dihydroethidium-derived oxidation products by HPLC in the assessment of superoxide production and NADPH oxidase activity in vascular systems. Am J Physiol Cell Physiol.
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