Hydrogen Peroxide Luciferin

Description:
Hydrogen peroxide (H$_2$O$_2$) is a component of cell signaling pathways that are necessary for the growth, development, and fitness of living organisms. Hydrogen Peroxide Luciferin is a chemoselective bioluminescent probe for the real-time detection of H$_2$O$_2$ in cell culture and living animals. Hydrogen Peroxide Luciferin is a boronic acid-caged firefly luciferin molecule that selectively reacts with H$_2$O$_2$ to release firefly luciferin, which triggers a bioluminescent response in the presence of firefly luciferase. The high sensitivity and selectivity of Hydrogen Peroxide Luciferin for H$_2$O$_2$, combined with the favorable properties of bioluminescence for in vivo imaging, afford a unique technology for real-time detection of basal levels of H$_2$O$_2$ generated in healthy, living mice.

Selective and concentration-dependent bioluminescent detection of H$_2$O$_2$ by hydrogen peroxide luciferin probe (HPL). (A) Total bioluminescent signal, integrated over 45 min, from PCL-1 (5 μM) alone (light gray bars) or incubated with various ROS (100 μM) or H$_2$O$_2$ (100 μM) and catalase (0.4 mg/mL) for 5, 20, 40, or 60 min. Signals were normalized to signal from HPL in the absence of any ROS. (B) Total bioluminescent signal, integrated over 45 min, from 5 μM HPL incubated for 1 h with increasing concentrations of H$_2$O$_2$ (0–250 μM). Signals were normalized to signal from HPL in the absence of H$_2$O$_2$. To quantify free luciferin formation in A and B, 100 μg/mL luciferase in 50 mM Tris buffer with 10 mM MgCl$_2$, 0.1 mM ZnCl$_2$, and 2 mM ATP (pH 7.4) was added to the HPL plus ROS solutions.

Bioluminescent signal from HPL in FVB-luc$^+$ mice. (A) Representative image (30 min postinjection) for mice injected with HPL (i.p., 0.5 μmol in 50 μL of 1:1 DMSO-PBS) immediately prior to injection of H$_2$O$_2$ (i.p., 0, 0.37, 1.5, 6, or 24 mM, left to right, in 100 μL of PBS). (B) Total photon flux, integrated over 1 h, for mice injected with HPL ± H$_2$O$_2$. H$_2$O$_2$ concentrations represent the H$_2$O$_2$ concentration in the i.p. cavity based on a total injection volume of 150 μL. Statistical analyses were performed with a two-tailed Student’s t test. **P < 0.005 (n = 5) and error bars are ± SD. (C) Representative image (12 min postinjection) for mice injected with HPL (i.p., 0.5 μmol in 50 μL of 1:1 DMSO-PBS) immediately following NAC (i.p., 0 or 0.2 mg in 100 μL PBS). (D) Total photon flux, integrated over 1 h, for mice injected with HPL ± NAC.

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Imaging and Applications:

- Imaging of hydrogen peroxide fluxes in cell culture and in living animals *in vivo*
- High sensitivity and low background to noise ratio
- Recommended imaging time is 10-60 min post injection of the probe
- Recommended dose is 0.5 uM per mouse injected i.p.

References: