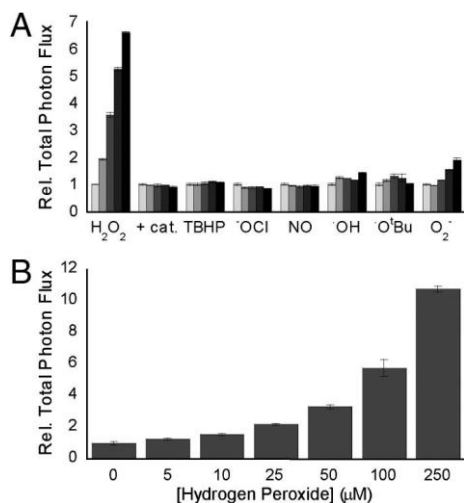


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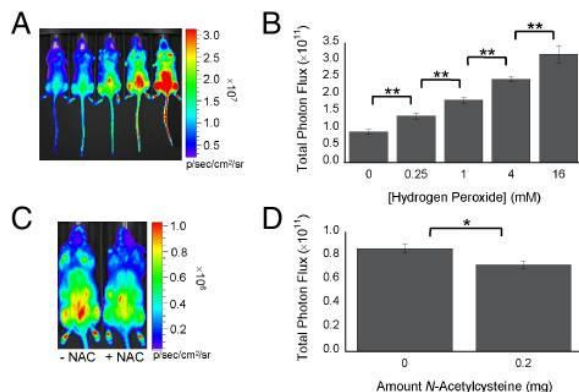
## Hydrogen Peroxide Luciferin

### Description:

Hydrogen peroxide ( $H_2O_2$ ) 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  $H_2O_2$  in cell culture and living animals. Hydrogen Peroxide Luciferin is a boronic acid-caged firefly luciferin molecule that selectively reacts with  $H_2O_2$  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  $H_2O_2$ , combined with the favorable properties of bioluminescence for in vivo imaging, afford a unique technology for real-time detection of basal levels of  $H_2O_2$  generated in healthy, living mice.



Selective and concentration-dependent bioluminescent detection of  $H_2O_2$  by hydrogen peroxide luciferin probe (HPL). (A) Total bioluminescent signal, integrated over 45 min, from PCL-1 (5  $\mu M$ ) alone (light gray bars) or incubated with various ROS (100  $\mu M$ ) or  $H_2O_2$  (100  $\mu M$ ) 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  $\mu M$  HPL incubated for 1 h with increasing concentrations of  $H_2O_2$  (0–250  $\mu M$ ). Signals were normalized to signal from HPL in the absence of  $H_2O_2$ . To quantify free luciferin formation in A and B, 100  $\mu g/mL$  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<sup>+</sup> mice. (A) Representative image (30 min postinjection) for mice injected with HPL (i.p., 0.5  $\mu mol$  in 50  $\mu L$  of 1:1 DMSO:PBS) immediately prior to injection of  $H_2O_2$  (i.p., 0, 0.37, 1.5, 6, or 24 mM, left to right, in 100  $\mu L$  of PBS). (B) Total photon flux, integrated over 1 h, for mice injected with HPL  $\pm H_2O_2$ .  $H_2O_2$  concentrations represent the  $H_2O_2$  concentration in the i.p. cavity based on a total injection volume of 150  $\mu L$ . Statistical analyses were performed with a two-tailed Student's *t* test.  $**P < 0.005$  ( $n = 5$ ) and error bars are  $\pm$  SD. (C) Representative image (12 min postinjection) for mice injected with HPL (i.p., 0.5  $\mu mol$  in 50  $\mu L$  of 1:1 DMSO:PBS) immediately following NAC (i.p., 0 or 0.2 mg in 100  $\mu L$  PBS). (D) Total photon flux, integrated over 1 h, for mice injected with HPL  $\pm$  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  $\mu$ M per mouse injected i.p.

### References:

1. Rhee SG.  $H_2O_2$ , a necessary evil for cell signaling. *Science*. 2006;312:1882–1883.
2. Stone JR, Yang S. Hydrogen peroxide: A signaling messenger. *Antioxid Redox Signaling*. 2006;8:243–270.
3. D'AutrEaux B, Toledano MB. ROS as signaling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol*. 2007;8:813–824.
4. Miller EW, Chang CJ. Fluorescent probes for nitric oxide and hydrogen peroxide in cell signaling. *Curr Opin Chem Biol*. 2007;11:620–625.
5. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nat Chem Biol*. 2008;4:278–286.
6. Paulsen CE, Carroll KS. Orchestrating redox signaling networks through regulatory cysteine switches. *ACS Chem Biol*. 2010;5:47–62.
7. Van de Bittnera GC, Dubikovskayaa EA , Bertozzia CR and Changa, CJ. In vivo imaging of hydrogen peroxide production in a murine tumor model with a chemoselective bioluminescent reporter. 21316–21321 | *PNAS* | December 14, 2010 | vol. 107 | no. 50