Robust chemiluminescent peroxidase substrates

Xie W, de Silva R, Eickholt RA, Mazelis ME, RA, Handley RS, Schaap AP, Akhavan-Tafti H
Lumigen, Inc., 22900 W. Eight Mile Rd., Southfield, MI 48083, USA

Chemiluminescent detection reagents for the widely used enzyme label horseradish peroxidase (HRP) in current use include those based on the oxidation of acridan substrates such as Lumigen® PS-1 and hydrazides such as luminol and isoluminol. These reagents must be supplied as two components and working solutions prepared daily because of limited storage stability. We report the synthesis of new, highly stable chemiluminescent peroxidase substrates and the development of enhanced detection reagents. Two compounds selected for commercial development, Lumigen PS atto for solution assays and Lumigen TMA-6 for solid phase assays, are structurally quite similar but possess subtle differences in performance properties. The substrates are readily water-soluble and stable to hydrolysis and peroxide. The robustness of the formulations allows storage as ready-to-use, single container working solutions for at least 3 weeks at room temperature and up to 4 months at 4 °C without degradation of performance. The reagents, based on an entirely new type of substrate, exhibits a linear response to peroxidase over five orders of magnitude. Measurement of plateau light intensity 6 min after addition of HRP to solutions of the reagent resulted in a linear calibration curve over the range 1.4 x 10⁻¹⁶ moles to 1.4 x 10⁻²¹ moles of enzyme. Chemiluminescence emission develops extremely rapidly compared to other substrates, reaching peak intensity in solution assays in under 1 min.