Applications of Lumigen PS-atto and TMA-6 chemiluminescent peroxidase substrates

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

Lumigen PS-atto and TMA-6 have been developed as enhanced chemiluminescent detection reagents for detection of horseradish peroxidase (HRP) conjugates in solution-phase and solid-phase immunodetection assays, respectively. These substrates suffer no deterioration of reagent performance on storage of the working solution up to three weeks at room temperature or four months at 4 °C. The structurally related compounds exhibit subtle differences in performance properties tailored to the intended application. Reaction of HRP leads to very rapid generation of peak light intensity. Unlike luminol-based reagents there is essentially no buildup time to peak emission. Light intensity was sufficiently intense to enable detection of HRP conservatively estimated at 10^{20} moles in a six minute assay with PS-atto and 10^{19} moles for TMA-6 with a linear dynamic range of at least four logs of peroxidase concentration. The extreme detection sensitivity allowed the development of highly sensitive enzyme immunoassays. A commercial colorimetric ELISA kit for TSH (Cobas Core, Roche) using an antibody-HRP conjugate was adapted for chemiluminescent detection by diluting the conjugate two-fold and substituting PS-atto for the detection reagent. The chemiluminescent assay achieved a measurement of 0.003 mIU/L with a signal/blank of 2, compared to a detection limit of 0.05 mIU/L the colorimetric assay. Lumigen TMA-6, developed for use in blotting applications such as western blotting, enjoys the advantages of PS-atto in rapid signal generation and reagent stability and permitted picogram level detection of proteins in membrane-based blotting assays. Signal duration is extended on typical blotting membranes to provide sufficient time for optimization of imaging parameters. A western blot assay of β-galactosidase achieved quantitation of 5 pg to 5 ng of protein at time points between 10 min and 2 hours.