

Chemiluminescence vs Chemifluorescence

Introduction

Traditionally, radioisotopic protocols have always been standard in the laboratory. But with costs and concerns associated with using and disposing of radioisotopes escalating, many researchers are turning to non-isotopic methods wherever possible. Consequently, new chemistries are being developed to substitute radio-isotopic methods. The most popular method currently is that of chemiluminescence although some scientists prefer chemifluorescence. So how do these differ and which is better?

Chemiluminescence

Chemiluminescence has become an accepted standard non-isotopic method for membrane applications. It is a chemical reaction occurring between an enzyme, such as horseradish peroxidase (HRP), and a chemiluminescent molecule, such as luminol, resulting in a light emission (Figure 1). This light emission can be detected with traditional x-ray films and CCD or PMT based detectors. It is more sensitive than colorimetric methods, the antibody-based systems provide target specificity and versatility, and the enzyme component provides signal amplification (Figure 2). With many manufacturers such as Pierce and GE Healthcare developing and optimising the chemistry involved in chemiluminescent detection, it has become an indispensable protocol for substituting radioisotopes in laboratories world-wide.

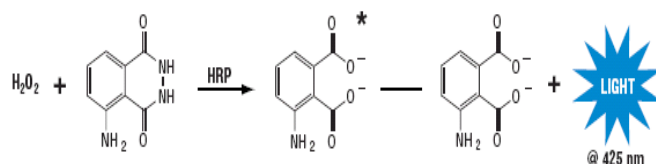


Figure 1- Chemiluminescence enzymatic reaction
Figure taken from Western blotting handbook, Thermo Scientific)

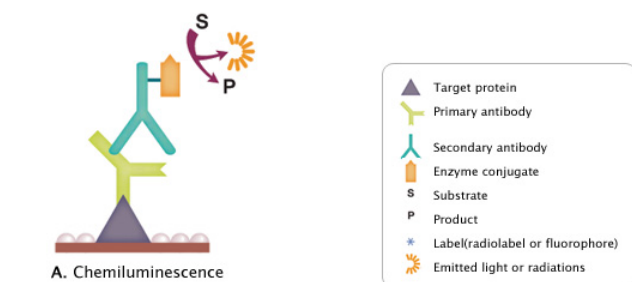


Figure 2- Schematic of chemiluminescence reaction

Chemifluorescence

Chemifluorescence is an alternative labelling and detection method for molecular biology and biochemistry that combines fluorescence and chemiluminescence. The difference between chemifluorescence and chemiluminescence lies in the detection mechanism. Whilst chemiluminescence generates light, based on an enzymatic reaction, chemifluorescence attaches a fluorescent molecule to either the secondary or tertiary antibody, which requires excitation via laser or some other high intensity light source (Figure 3). Once the fluorescent molecule is excited, it will generate a light emission that can be collected by a CCD or PMT based detector. It also provides greater sensitivity, target specificity and signal amplification than colorimetric methods as does chemiluminescence.

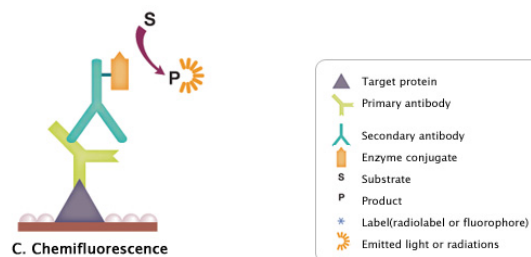


Figure 3- Schematic of chemifluorescence reaction

Overall, chemiluminescence is favoured over the two methods for the following reasons summarized in table I.

	CHEMILUMINESCENCE	CHEMIFLUORESCENCE
Detection system	CCD, X-Ray film	CCD
Excitation	None	Required
Detection levels	Femtogram (fg)	Femtogram (fg)
Shelf life	Up to 1 year	<1 month
Cost	Inexpensive	Expensive

Table I- Comparison of Chemiluminescence and Chemifluorescence

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