



SCIENCE IMAGING SYSTEMS

# Application Note

**No. 6**

## Fundamentals of Chemiluminescence Detection

LAS-1000

### Introduction

Chemiluminescence is the emission of light by a chemical reaction. In earlier chemiluminescent imaging methods, the extremely short lifetime of the chemiluminescence limited detection to use of high-performance liquid chromatography (HPLC). (Reference 1)

New technologies developed in recent years have greatly extended the utility of chemiluminescence, enabling detection by less demanding methods such as membrane blotting. In the detection method used up to now, visualization has involved holding the blotted membrane in contact with x-ray film for an exposure period of several decades of seconds to several hours.

Fujifilm now offers a new system, the LAS-1000, which is a cooled CCD camera system, capable of producing a digital image from even very weak luminescence. By digitizing the image, the LAS-1000 markedly facilitates quantitative image analysis and image presentation. In short, the LAS-1000 is a chemiluminescence detection system featuring outstanding resolution and sensitivity.

In this issue, the basic principles of chemiluminescence are examined together with a number of commonly used reagents.

### Contents

1. Typical Chemiluminescent Reagents and Their Mechanisms of Action
  - (1) Luminols
  - (2) 1,2-Dioxetanes
2. Popular Reagents
3. References

### Summary

- Luminols are commonly used as the substrate for chemiluminescence reaction in Western blot detection.
- Dioxetanes are commonly used as the substrate for chemiluminescence reaction in Southern blot detection.
- Many reagent kits are available for use with both Western blot and Southern blot detections.

LAS

# 1 Typical Chemiluminescent Reagents and Their Mechanisms of Action

As illustrated in Fig. 1-1, chemiluminescence is the emission of light from a chemically excited compound (whose molecules are in an electronically excited state) when it returns to the unexcited or ground state.

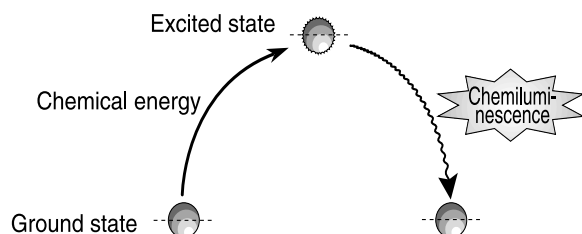


Fig. 1-1

## Excited state

Excitation is the process of a molecule's transition from its normal low-energy state to a high-energy (excited) state.

## Ground state

The normal low-energy state of a molecule

Fig. 1-1 The chemiluminescence mechanism

Luminols and 1,2-dioxetanes are two typical chemiluminescent substrate groups.

## (1) Luminols

The knowledge that luminol emits light by catalysis with the iron in blood dates back many years and has stimulated much research. In the presence of hydrogen peroxide, luminol decomposes through intermediates and emits light in the course of the decomposition. The technique of enhancing this emission by catalyzing the decomposition reaction with peroxidase, an enzyme, is also well known. In 1985, Kricka and coworkers discovered that iodophenol compounds are strong enhancers that intensify luminol chemiluminescence about 1000 times, while also prolonging the duration of chemiluminescence. This method, known as "enhanced chemiluminescence," has been further improved and refined in recent years. (References 2-4)

## Reaction Scheme

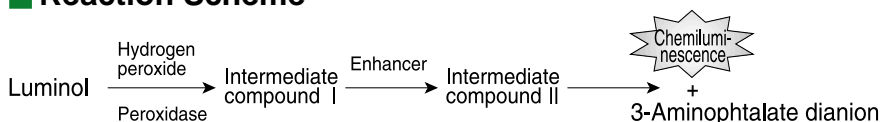


Fig. 1-2

Peroxidase-catalyzed oxidation of luminol by hydrogen peroxide produces Intermediate compound I. Intermediate compound I reacts with the enhancer to generate Intermediate compound II which, in turn, is converted into 3-aminophthalate dianion with the concomitant emission of light-chemiluminescence.

## Emission Spectrum

The chemiluminescence spectrum of the luminol reaction is shown below.

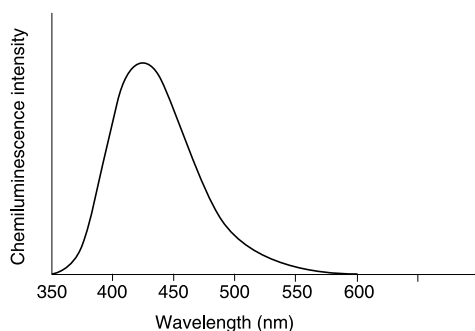
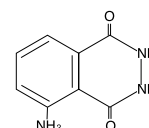


Fig. 1-3

## Chemical structure of luminol



## Peroxidase (POD)

An enzyme that catalyzes the reaction  $H_2O_2 + AH_2 \rightarrow 2H_2O + A$ . Horseradish peroxidase (HRP) is well known.

Fig. 1-2 Scheme of luminol chemiluminescence reaction

## Chemical structure of 3-aminophthalate dianion

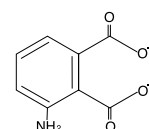


Fig. 1-3 Chemiluminescence spectrum of luminol

## ■ Time Course of Luminescence

The luminescence intensity peaks in 5-20 minutes and then decreases with a half-life of 60 minutes.

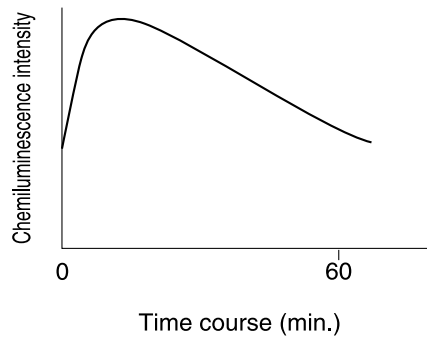


Fig. 1-4

Fig. 1-4 Time course of ECL™ chemiluminescence

## ■ Application: Detection of Western Blot

Western blotting involves electrophoresis of proteins in SDS-PAGE gels and then transferring the separated proteins to a PVDF or other membrane filter by means of an electrical field. Antigen-antibody reaction or avidin-biotin reaction is utilized for specific detection of the target protein.

The image below is an ECL™ detection of rat brain P2 membrane protein after blotting, antigen-antibody reaction and enzyme-labeling. Fig. 1-6 shows the overall scheme of the reaction on the membrane filter.

**PVDF (polyvinylidene fluoride)**  
A highly hydrophobic membrane used for protein blotting after prewetting

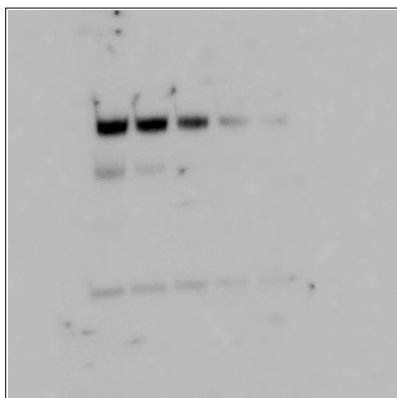


Fig. 1-5

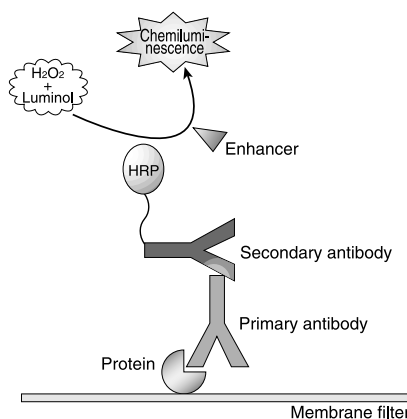


Fig. 1-6

Fig. 1-5 Chemiluminescent detection of Western blot

Sample: Rat brain P2 membrane protein

\* Sample by courtesy of Mitsubishi Kasei Institute of Life Sciences

Fig. 1-6 Schematic diagram of Western blot detection on membrane filter

## (2) 1,2-Dioxetanes

In 1989 Bronstein and coworkers reported a new 1,2-dioxetane-based chemiluminescent alkaline phosphatase substrate (AMPPD<sup>®</sup>) that has since attracted attention for its high-sensitivity. Although AMPPD<sup>®</sup> is stable in aqueous solution, it decomposes into an unstable intermediate compound and emits light when hydrolyzed by alkaline phosphatase (ALP).

A recent application of 1,2-dioxetane-based chemiluminescent substrates is in Southern hybridization detection in the field of molecular biology. Use of CDP-*Star*<sup>®</sup>, a newly developed dioxetane-based substrate with higher luminescence intensity than either AMPPD<sup>®</sup> or CSPD<sup>®</sup>, is increasing rapidly. (References 5-8)

### Reaction Scheme



Fig. 1-7

A chemiluminescent substrate, AMPPD<sup>®</sup>, reacts with ALP to generate an intermediate compound that spontaneously splits into adamantanone and a fluorophor. The fluorophor is in a chemically excited state and emits light on return to its ground state.

### Emission spectrum

The chemiluminescence spectrum of the CDP-*Star*<sup>®</sup> reaction is shown below.

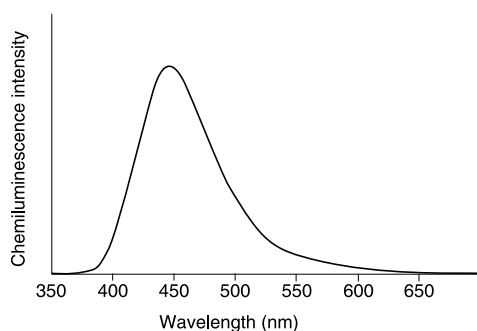
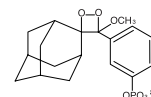


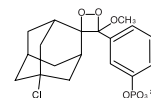
Fig. 1-8

### Chemical structures:

#### AMPPD<sup>®</sup>



#### CSPD<sup>®</sup>



#### CDP-*Star*<sup>®</sup>

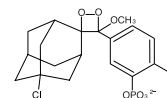


Fig. 1-7 Scheme of 1,2-dioxetane-based substrate chemiluminescence reaction

### Alkaline phosphatase (ALP)

An enzyme that cleaves phosphate in alkaline condition

Fig. 1-8 Chemiluminescence spectrum by CDP-*Star*<sup>®</sup> on nylon membrane

## ■ Time Course of Luminescence

As shown by the time-course curves below, the chemiluminescence of the CDP-*Star*<sup>®</sup> reaction continues for more than 24 hours. Quicker response and stronger emission than either AMPPD<sup>®</sup> or CSPD<sup>®</sup> make CDP-*Star*<sup>®</sup> an excellent substrate.

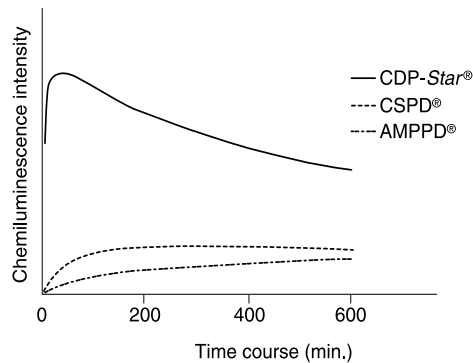


Fig. 1-9

Fig. 1-9 Time course of 1,2-dioxetane chemiluminescence

## ■ Application: Detection of Southern Blot

Southern blotting involves transferring electrophoretically separated DNA fragments from a gel to a membrane filter. A labeled probe is used for specific detection of DNA on the membrane.

Fig.1-10 results from the use of CDP-*Star*<sup>®</sup> to detect genomic DNA of knock-out mouse after blotting to nylon membrane. Fig. 1-11 schematically illustrates the reaction on the membrane filter.

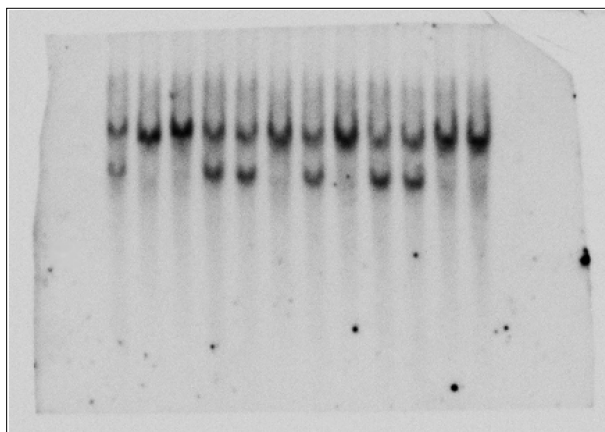


Fig. 1-10

Fig. 1-10 Chemiluminescent detection of Southern blot

Sample: Genome DNA of knock-out mouse by DIG-labeled probe

\* Sample by courtesy of Mitsubishi Kasei Institute of Life Sciences

DIG: Digoxygenine

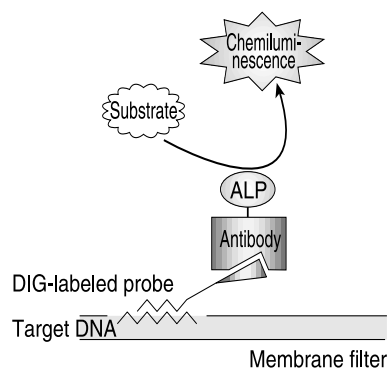


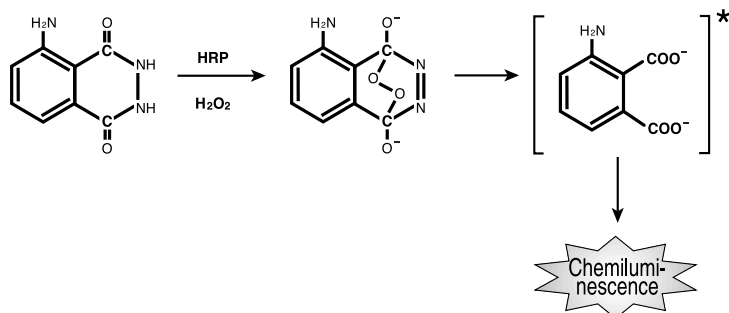
Fig. 1-11

Fig. 1-11 Schematic diagram of Southern blot detection on membrane filter

## 2 Popular Reagents

### ■ Peroxidase

#### Luminols

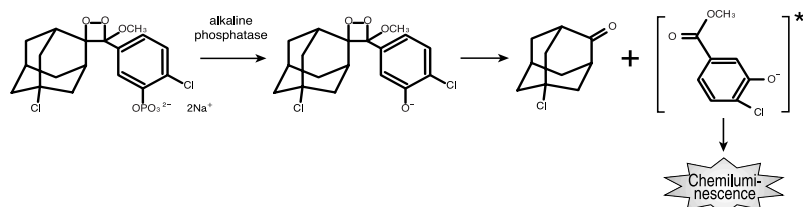


#### Reagent Kits

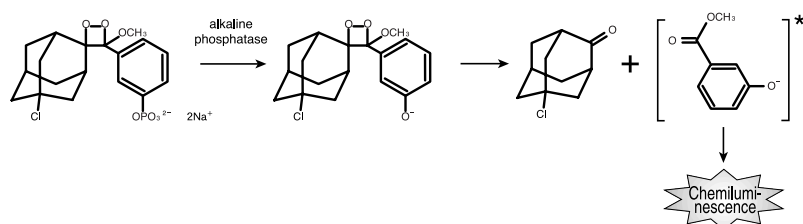
Product	Sample	Distributor
ECL™ Direct Nucleic Acid Labelling and Detection Systems	DNA,RNA	
SuperSignal® Chemiluminescent Substrate Kit	DNA,RNA	
BM Chemiluminescence Western Blotting Substrate(POD)	Protein	
ECL™ Western Blotting Detection Reagents	Protein	
SuperSignal® CL-HRP Substrate System	Protein	

## ■ Alkaline phosphatase

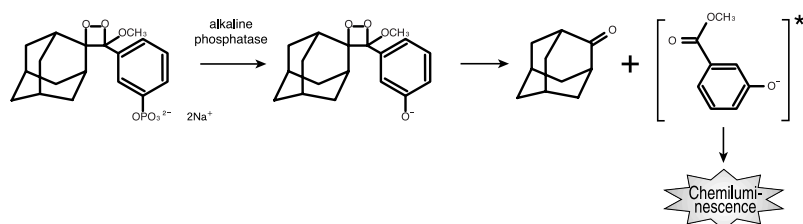
### CDP-Star® (C<sub>18</sub>H<sub>19</sub>Cl<sub>2</sub>O<sub>7</sub>PNa<sub>2</sub>)



### CSPD® (C<sub>18</sub>H<sub>20</sub>ClO<sub>7</sub>PNa<sub>2</sub>)



### Lumigen® PPD (C<sub>18</sub>H<sub>21</sub>O<sub>7</sub>PNa<sub>2</sub>)



## Reagent Kits

Product	Sample	Substrate	Distributor
DIG Luminescent Detection Kit	DNA, RNA	CSPD®	
Gene Images™ Labelling and Detection Systems	DNA, RNA	CDP-Star®	
Gene-Lite® Chemiluminescence Detection Kit	DNA, RNA	CDP-Star®	
PHOTOGENE™ Nucleic Acid Detection Systems Version 2.0	DNA, RNA	Lumi-Phos®530	
Phototope™-Star Western Blot Detection Kit	Protein	CDP-Star®	

### 3 References

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