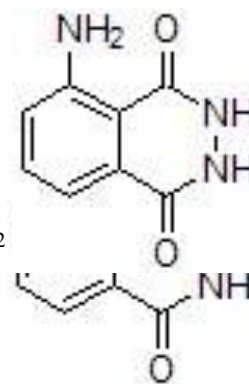


## Luminol

*Chemiluminescent substrate to detect peroxidase (immunoassays, cell assays),  
and a great probes of metal ion-mediated oxidative events*

### Products Description

<b>Name :</b>	<b>Luminol, FluoPure Grade</b> 3-AminoPhthalHydrazide ; CAS: 521-31-3
<b>Catalog Number :</b>	FP-57578A, 1 g <b>MW: 177.16</b> <sup>(Z)</sup> Purity: min. 95.0 % . UV specifications on inquire.
<b>Solubility:</b>	Good in DMF. poorly in water; 2 % in 1N NaOH; 50 mg in 2-propanol/ammonia/water 7:1:2
<b>Absorption / Emission :</b>	$\lambda_{exc}/\lambda_{em}$ (methanol) : 355/413nm greatest relative intensity at 425 nm, with optimal pH 9-10.3
<b>EC (Extinction coefficient)</b>	7 650 M <sup>-1</sup> cm <sup>-1</sup> (end product) pale yellow to tan to greenish powder



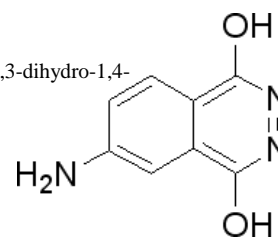
**Xi**  
**R36/37/38**

<b>Name :</b>	<b>Luminol</b> 3-AminoPhthalHydrazide
<b>Catalog Number :</b>	FP-04247A, 1g FP-04247B, 5g FP-04247C, 25g FP-04247G, 100g

<b>Name :</b>	<b>Luminol, Na salt</b> 3-Amino-phthalhydrazide Na salt; CAS: 20666-12-0
<b>Catalog Number :</b>	FP-CA9611 2.5g <b>MW: 199.15</b> <sup>(Z)</sup> Purity: min. 95.0 %

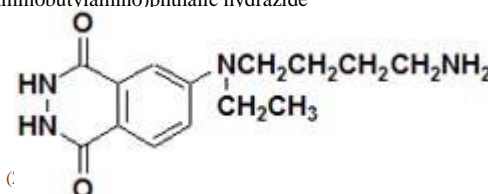
<b>Name :</b>	<b>Luminol, HCl</b> 3-Amino-phthalhydrazide HydroChloride; CAS: 74165-64-3]
<b>Catalog Number :</b>	FP-BG076 <b>MW: 213.62</b>

<b>Name :</b>	<b>Isoluminol, monohydrate</b> 4-Aminophthalhydrazide monohydrate; -(N-(4-aminobutyl)-N-ethylamino)-2,3-dihydro-1,4-phthalazinedione] ,, [4-(N'-ethyl-4-aminobutylamino)phthalic hydrazide CAS: 3682-14-2
<b>Catalog Number :</b>	FP-07624A-, 1g <b>MW: 195.18</b> <sup>(M)</sup> Purity: ≥97% Soluble in DMSO, DMF and CH <sub>3</sub> OH



**Xi**  
**R36/37/38**

<b>Name :</b>	<b>Isoluminol ABEI</b> 4-AminoButyl)-N-EthylIsoLuminol; 4-Aminophthalhydrazide monohydrate; -(N-(4-aminobutyl)-N-ethylamino)-2,3-dihydro-1,4-phthalazinedione] ,, [4-(N'-ethyl-4-aminobutylamino)phthalic hydrazide CAS: 66612-29-1
<b>Catalog Number :</b>	FP-60404A, 5mg <b>MW: 276.34</b> <sup>(M)</sup> Purity: ≥97%



**Storage:** Room temperature; Protect from air, light and moisture

## Introduction

**Luminol** is a chemiluminescent substrate for horseradish peroxidase (HRP, PO). It also allow for sensitive detections of oxidative species.

- HRP is a common marker enzyme for antibodies, it can catalyse the chemiluminescent oxidation of luminol in the presence of hydrogen peroxide. This transformation is used in ultra-sensitive ELISA assays. Luminescence can be detected at very low levels using extremely sensitive scintillation counters. Luminol is also used for the many other detection systems, coupling HRP/H<sub>2</sub>O<sub>2</sub> reaction with other enzymatic systems.
- Luminol is used as a blood stain in criminal investigation. For this luminol is mixed with a liquid containing hydrogen peroxide. If the mixture comes in contact with hemoglobin, the iron in the hemoglobin accelerates chemiluminescent oxidation of luminol.
- Luminol also serve as a sensitive chemiluminescent probes for metal ion-mediated oxidative events. However for live cells, [MCLA \(FP-38544A\)](#) is recommended.

**Isoluminol** is an amino-coupling reagent for protein detection that is also chemiluminescent which may prove better than radioimmunoassay methods.

## Guidelines for use– Luminol

### Handling and Storage

Luminol is stored at room temperature under inert gaz, protected from light and heat.

Luminol is relatively insoluble in water, but is quite soluble in base (sat 200mg/ml). The sodium salt (FP-CA9611) is readily soluble in water.

### HRP immunoassays <sup>(r)</sup>

The sodium salt and free base forms of luminol undergo photochemical changes resulting in the formation of a series of compounds which are significantly inhibitory to enhanced chemiluminescence. As Luminol is thermally unstable, luminol eagent should be protected from light and high temperature.

Luminol is readily oxidized in basic solution, releasing of energy as visible light. The reaction can be carried out in a variety of media including water, and aprotic solvents (DMSO or DMF). The mechanism of oxidation varies with the solvent, and slightly different conditions are needed. In aprotic media, only molecular oxygen and a strong base are needed to produce chemiluminescence ( $\lambda_{\text{max}} = 485 \text{ nm}$ ). In aqueous systems, a strong base, either molecular oxygen or a peroxide and an auxiliary oxidant such as hypochlorite or perborate are required for chemiluminescence ( $\lambda_{\text{max}} = 425 \text{ nm}$ ). The actual form that emits light is the aminophthalate ion [\(Shakashiri 1983\)](#).

Applications include:

- Microestimation of glucose and glucose oxidase using enzyme-induced chemiluminescence [\(Bostick 1975, Puget 1976\)](#)
- Inhibitor of poly ADP Ribose Synthase [\(Banasik 1989\)](#)
- Demonstrations of the phenomenon of chemiluminescence, using different sensitizers to produce different colors of light [\(stott 1987\)](#)

Other protocols may found in the literature.

### Test for blood [\(Laux 1991\)](#)

As Sodium perborate (3.5 g) to 500 mL distilled water and dissolve thoroughly.

Add Sodium carbonate (25 g) and luminol (9.5 g) and dissolve. Stand solution for five minutes to allow undissolved chemicals to settle and decant into a plastic spray bottle and is ready to use.

Test: applied the luminol reagent as a fine mist on the surface to be tested. True blood stains will luminesce with an even glow that will last for several seconds; observe in as dark as possible for better viewing.

This test is only presumptive, since it is the iron in the heme which catalyzes the oxidation and subsequent light emission. The presence of copper as a contaminant will accelerate oxidation. Try the spray on a freshly cleaned copper penny.

Other protocols may found in the literature.

FT-57578A

## Analytical test for copper - Luminol

-Prepare the reagent:

Dissolve 0.1 g luminol in 10 mL concentrated ammonia and dilute with 100 mL water.

Before use, dilute 1mL of this solution with 4 mL distilled water and mix with 0.5 mL of 3% H<sub>2</sub>O<sub>2</sub>.

-Test:

To 0.5 mL of this reagent solution in a small test tube, add the test solution containing iron(II) or copper(II).

This luminol reagent glows with a blue-violet luminescence in the presence of as little as 0.13 mg of copper or 0.25 mg of iron. The dilution limit is 1:2 500 000. A standard curve established with a photometer permits quantitative determination.

Other protocols may be found in the literature. Alternative method using [BC Assay UP40840A](#).

## Biology assays Assays with Oxidative species <sup>(r)</sup>

Please refer to the literature

## References - Luminol

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- Roswell D.F., White E.H., The chemiluminescence of luminol and related hydrazides. *Methods Enzymol.* 1978, 57, 409
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- Laux, Dale L., *Law Enforcement Technology*, May, 26-27 (1991) [Blood test]
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- Beranger A. et al.; Synthesis and chemiluminescence of new derivatives of isoluminol; *Da.n . J. Chem.* 65, 1392 (1987); [Article](#) [Isoluminol and ABEI / Luminescence reaction kinetics]
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- *Nucleic Acids. Res* 20, 5061 (1992) *Biochem. Mol. Biol. Int.* 33, 1179 (1994)
- *Free Radic. Biol. Med.* 6, 623 (1989) *J. Immunol.* 129, 1589 (1982)

## References - Isoluminol

- *Methods Enzymol.* 57, 424 (1978)
- J.S.Simpson, et. al., *Nature* 279, 646 (1979).

## Additional information

## Related products

- UptiLight Substrates for HRP
  - ELISA: High sensitivity, UltraSensitivity #996201
  - Blotting: Classic, Spray, High sensitivity, UltraSensitivity 58372A
- Peroxidase from HorseRadish #UP146500
- Hydrogen Peroxide #15983
- Lucigenin #FP-46915A & MCLA #FP-38544A
- ABEI, arninohexylethylisoluminol

## Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com/>

Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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