

5-AMINO-2,3-DIHYDRO-1,4-PHTHALAZINEDIONE Sigma Prod. No. A8511

ProductInformation

CAS NO.: 521-31-3

SYNONYMS: Luminol; 3-aminophthalhydrazide; o-

aminophthaloyl hydrazide

PHYSICAL DESCRIPTION:

Appearance: Yellow powder, occasionally with a tan or greenish

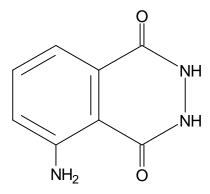
cast.

Molecular formula: C₈H₇N₃O₂ Molecular weight: 177.2 Melting point: 319-320EC²

The chemiluminescence spectrum of luminol indicates greatest

relative intensity at

425 nm, with optimal pH 9-10.3



STABILITY / STORAGE AS SUPPLIED:

Luminol is stable at room temperature if stored protected from light. It should be re-evaluated for suitability in user application every three to five years.

SOLUBILITY / SOLUTION STABILITY:

Sigma tests the free acid at 50 mg/mL in DMSO, obtaining a clear yellow solution. 1

Luminol free acid is comparatively insoluble in water, but is quite soluble in base. (Saturation is approximately 200 mg/mL.⁵ A4685, the sodium salt, is readily soluble in water.)¹ Solutions are very sensitive to light and the presence of metal cations; typically they are only stable 8-12 hours.⁴

"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." Luminol was also shown to be thermally unstable, so luminol and its solutions should be protected from light and high temperature.

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GENERAL USAGE:

Luminol is readily oxidized in basic solution, with the release of energy as visible light. The reaction can be carried out in a variety of media including protic solvents such as 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 (λ_{max} = 485 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 (λ_{max} = 425 nm). The actual form that emits light is the aminophthalate ion.³

Applications include:

- Microestimation of glucose and glucose oxidase using enzyme-induced chemiluminescence 6,7
- Inhibitor of poly ADP Ribose Synthase⁸
- Demonstrations of the phenomenon of chemiluminescence, using different sensitizers to produce different colors of light.⁵
- Presumptive test for blood^{4,9}

Sodium perborate (3.5 g of Aldrich product number 24,412-0) is added to 500 mL distilled water and thoroughly dissolved. Sodium carbonate (25 g) and luminol (9.5 g) are then added and dissolved. The solution is allowed to stand for five minutes to allow any undissolved chemicals to settle. The solution is then decanted into a plastic spray bottle and is ready to use. It should be applied as a fine mist on the surface to be tested. True bloodstains will luminesce with an even glow that will last for several seconds; for better viewing, the scene should be as dark as possible.

It is significant to note that 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.

- Analytical test for copper⁹

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GENERAL USAGE: (continued)

Dissolve 0.1 g luminol in 10 mL concentrated ammonia and dilute with 100 mL water. Before use, dilute 1 mL of this solution with 4 mL distilled water and mix with 0.5 mL of 3% H_2O_2 . To 0.5 mL of this reagent solution in a small test tube, add the test solution containing iron(II) or copper(II). This mixture glows with a blue-violet luminescence in the presence of as little as 0.13 μ g of copper or 0.25 μ g of iron. The dilution limit is 1:2,500,000. A standard curve established with a photometer permits quantitative determination.

CITED REFERENCES:

- 1. Sigma quality control.
- 2. Merck Index, 12th Ed., #5628 (1996).
- 3. Shakhashiri, Bassam Z., Chemical Demonstrations, VOL. 1, 156-167 (Univ. of Wisconsin Press, 1983).
- 4. Laux, Dale L., Law Enforcement Technology, May, 26-27 (1991).
- 5. Stott, R.A.W. and Kricka, L.J., *Biolumin. Chemilumin., Proc. Int. Biolumin. Chemilumin. Symp.*, 4th (1987), Meeting Date 1986, 237-240. ed. Schoelmerich, J. (Wiley Press, Chichester, UK).
- 6. Bostick, D.T. and Hercules, D.M., Analytical Chemistry, 47, 447-451 (1975).
- 7. Puget, K. and Michelson, A.M., Biochemie, 58, 757-758 (1976).
- 8. Banasik et al., "New Inhibitors of Poly ADP Ribose Synthase", in ADP Transfer Reactions, ed. Jacobson & Jacobson (Springer-Verlag Press, 1989).
- 9. Sigma's Forensic Dept.
- 10. Sigma supplier files.

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