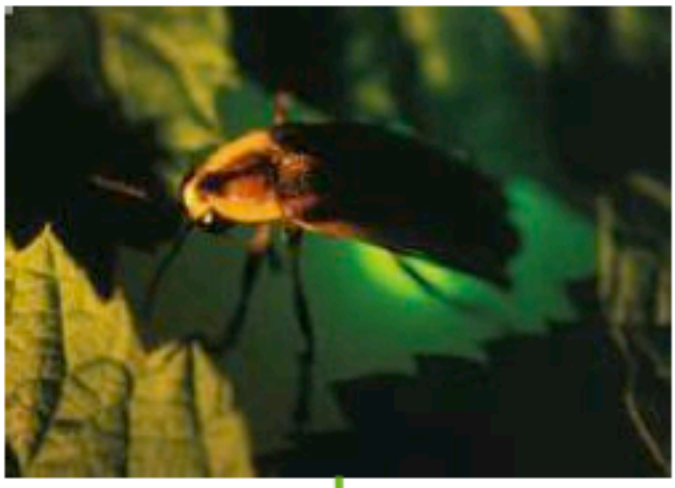
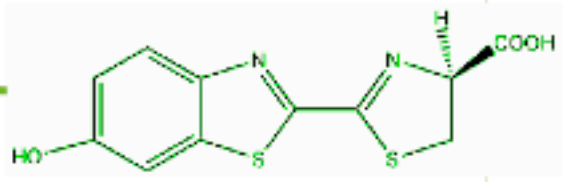
PRODUCT CATALOGUE





RESEM

Content:

WELCOME to RESEM

During the last 15 years **RESEM** has become specialized in organic and enzymatic multi-step synthesis and now carries **a small, but highly sophisticated range of products**.

New technologies, specifically developed for **post-synthesis purification** have lead to the highest quality and purest products available on the market today.

And that is why **RESEM** key products **Photinus D- and L-Luceferin and derivates** as well as a state-of-the-art range of **other synthesized biochemical substrates like Coelenterazine** are presently used by leading global companies for research.

And has also made **RESEM** the global supplier for major pharmaceutical companies.

Prompt delivery from stock or tailor-made consignment contracts are guaranteed to reduce the customer's inventory costs creating added financial value.

Furthermore **RESEM customers** have appreciated the dedicated personal service and the fast response to their particular needs.

For further inquiries please use this contact form or call +31 23 5552502 and ask for René Hiensch.

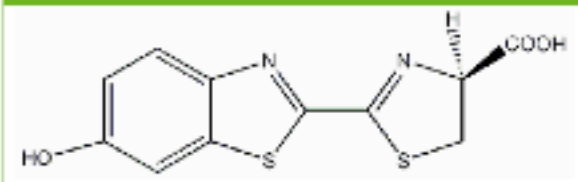
Introduction D - & L-Luciferin, - Na salt, - K salt

Published procedures for the synthesis of D-Luciferin are complicated, involve several steps and result in formation of side-products which are strong inhibitors of firefly luciferase. The RESEM D-Luciferin is synthesized by a new route including a novel purification method whereby side-products are efficiently removed.

The entire synthetic procedure is well suited for large scale production, which makes it possible to offer this product at very competitive prices. The end-product is vacuum-dried and stored under argon to ensure maximum stability. The product is available in amounts of 100, 500 and 1000 mg. Other quantities can be arranged provided a longer delivery time is accepted.

Product Information D- & L-Luciferin, - Na salt, - K salt

D-Luciferin

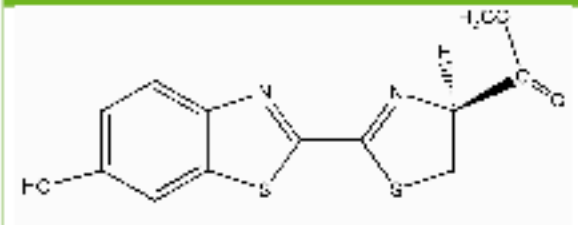


$C_{11}H_8N_2O_3S_2$
Mol.Wt: 280.32

Specifications D-Luciferin

- Appearance: yellowish powder
- Stocking: at -18°C in dark and dryness
- Water content: <0.05%
- ATP, nucleases and proteases not detectable
- Contains 0.05% acetic acid as an antistatic
- Optical purity: >99% by HPLC
- Chemical purity: >99% by HPLC
- Packed under argon, shipped refrigerated

D-Luciferin Methyl ester

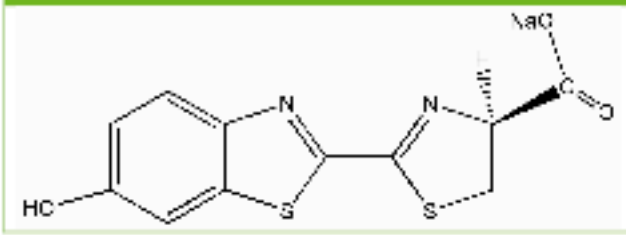


$C_{12}H_8N_2O_3S_2$
Mol.Wt: 294.35

Specifications D-Luciferin methyl ester

- Appearance: light brownish powder
- Stocking : at -18°C in dark and dryness
- Water content: <0.05%
- ATP, nucleases and proteases not detectable
- Contains 0.05% acetic acid as an antistatic
- Optical purity: >99% by HPLC
- Chemical purity: >99% by HPLC
- Packed under argon, shipped refrigerated

D-Luciferin Sodium salt

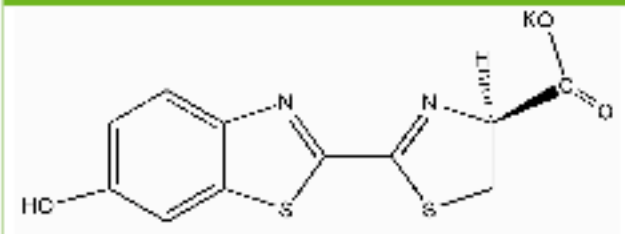


$C_{11}H_7N_2NaO_3S_2$
Mol.Wt: 302.3

Specifications D-Luciferin Na salt

- Appearance: white dense powder, readily soluble in water
- Stocking: at -18°C in dark and dryness
- Water content: <0.05%
- ATP, nucleases and proteases not detectable
- Optical purity: >99% by HPLC
- Chemical purity: >99% by HPLC
- Packed under argon, shipped refrigerated

D-Luciferin Potassium salt



$C_{11}H_7KN_2NaO_3S_2$
Mol.Wt: 318.4

Specifications D-Luciferin K salt

- Appearance: Yellowish powder
- Stocking: at -18°C in dark and dryness
- Water content: <0.05%
- ATP, nucleases and proteases not detectable
- Optical purity: >99% by HPLC
- Chemical purity: >99% by HPLC
- Packed under argon, shipped refrigerated

Chemical Purity

QUALITY CONTROL PROCEDURES - HPLC analysis of chemical purity

The biochemical performance of each lot of D-luciferin is assayed at 25°C by an automatic procedure using a special quality control kit containing standardised preparations of all components (luciferase, ATP, buffer etc.) required for the measurement. Light emission is measured at the following final concentrations of D-luciferin: 0.1 mg/mL (below optimum), 0.2 mg/mL (optimum) and 0.3 mg/mL (above optimum). Each run begins and ends with 0.2 mg/mL of a reference lot of D-luciferin. Assays are performed in triplicates for each sample.

The analysis is performed with Shimadzu SPD-10A UV Spectrometric Detector in Shimadzu Liquid Chromatograph. The procedure uses a buffered-reverse phase technique with a gradient elution. An example of a chromatogram obtained with Lot 1035 at 330 nm is shown in Fig. 1. Results from 5 samples performed in duplicates are summarized in Table 1. Results obtained at 265 nm were similar. In the interval 220-540 nm no additional chromatographic peaks were detected. The average retention time of D-Luciferin was 7.126 ± 0.013 min.

Peak no.2 at 9.302 ± 0.021 min corresponds to deoxyluciferin. The average percent purity for Lot 1035 is 99.65 ± 0.03% D-Luciferin. According to specifications, the percent purity of D-Luciferin (free acid) must be greater than 99.5%.

HPLC analysis of chemical purity

Fig. 1: Chromatogram of D-Luciferin (Lot 1035) at 330 nm. Insert shows absorbance using an enlarged scale.

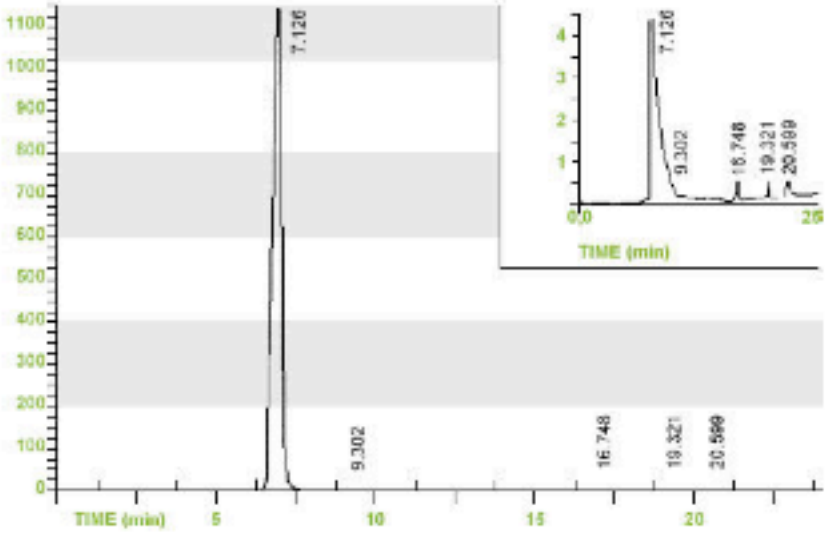


Table 1: Chemical purity analysed by HPLC. UV detection at 330 nm

Retention time	Area (%)
7.126	99.68
9.302	0.02
16.748	0.10
19.321	0.06
20.599	0.14

Optical Purity

QUALITY CONTROL PROCEDURES - HPLC analysis of optical purity

The analysis is performed by a method using the same HPLC equipment as described above. An example of a chromatogram obtained with Lot 1035 is shown in Fig. 2. Results from 5 samples performed in duplicates are summarized in Table 2. According to experiments with pure D- and L-Luciferin assayed alone and in mixtures the average retention time for L-Luciferin is 2.622 ± 0.062 min and for D-Luciferin 5.011 ± 0.2019 min. For Lot 2019 the first two peaks correspond to D- and L-Luciferin, respectively. Additional three peaks were found in all 10 chromatograms. These peaks (no. 3, 4 and 5) had the retention times: 7.286 ± 0.041 min, 7.768 ± 0.241 min and 9.325 ± 0.089 min.

In Table 2 the D- and L-Luciferin levels would be estimated as 0.17 ± 0.02% and 99.51 ± 0.05%. The chemical purity was 99.68% (cf. Table 2) from which 0.17% L-Luciferin should be subtracted to give a D-Luciferin purity (chemical and optical) of 99.5%. According to specifications the L-Luciferin level must be lower than 0.7%.

HPLC analysis of optical purity

Fig 2: Chromatogram of D-Luciferin (Lot.2019) at 220 nm. Insert shows absorbance using an enlarged scale.

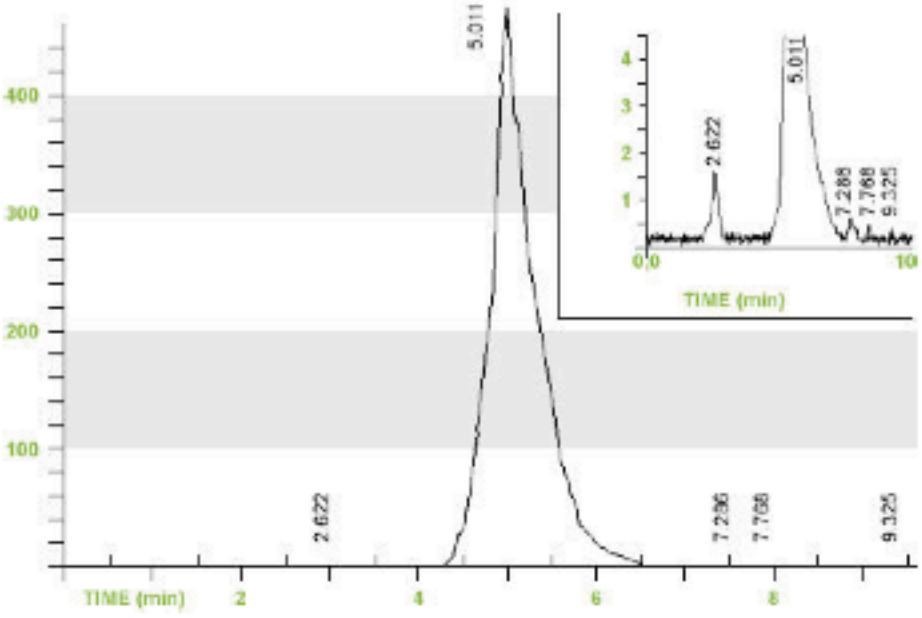


Table 2: Optical purity analysed by HPLC. UV detection at 220 nm

Retention time	Area (%)
2.622	0.17
5.011	99.51
7.286	0.16
7.768	0.10
9.325	0.06

The pure form of D-Luciferin is stable for five years when stored dry at -18°C under an inert gas like argon or nitrogen. Alkaline pH and contact with oxygen deteriorates the product rapidly. The corresponding L- Luciferin, suitable as signal prolonger for "glow form" bioluminescence is also available.

The recommended use of D-Luciferin in ATP assay vary in details dependent if it is used in cell proliferation or in bioluminescent assays. It is important a suitable concentration of magnesium is available for the subsequent light production.

Chemical and optical purity are analyzed by reverse phase and chiral chromatography, respectively. The UV detection at 330 nm is performed with Shimadzu SPD-10A UV Spectrometric Detector.

Performance

QUALITY CONTROL PROCEDURES - Biochemical performance

The biochemical performance of D-Luciferin is assayed at 25°C by an automatic procedure using a Berthold Luminometer. The assay is based on standardized preparations of all components (luciferase, ATP, buffer etc.) required for the measurement. The light emission values obtained with 1.000 and 0.250 mg/mL of D-Luciferin and compared with a reference lot of D-Luciferin.

Actual results for 5 samples from a typical lot, in the absence of substrate inhibition and non-competitive inhibitors, will approach or exceed 100%. With test and reference lots of similar qualities a value around 100% is expected.

Table 1: Biochemical performance of D-Luciferin

Reference us used in concentration of 0.200 mg/mL	Test 0.200 mg/mL
Lot No.1021	101.0 ± 1.9%
Specification	>97.0%

DISSOLVING D-Luciferin

Warm Luciferin substrate reagent to room temperature before starting.

D-luciferin (acid form) can be dissolved at a concentration of 10 mg/mL (35.7 mmol/L) in an equimolar concentration of base (pH 7.6 to 7.8), e. g. Tris – Tricine and Tris EDTA and HEPES buffers.

A solution is readily obtained using stoichiometric amounts of diluted $NaHCO_3$ solution. Add 100 mg Luciferin into 3 mL of 1% $NaHCO_3$ in a buffer. Vortex to obtain immediately a clear yellow solution which should be protected from direct light exposure.

STABILITY

D-Luciferin is sensitive to light and oxygen before as well after dissolving. If the vial containing the solid form of D-Luciferin should be opened and closed again, it is recommended to fill the vial with an inert gas before closing the lid. Argon has a higher density than air. This makes it more convenient to fill the vial with argon as compared to nitrogen.

The solid form of D-Luciferin should be protected from light and stored at -20°C. Solutions of D-Luciferin should be protected from light and stored at -80°C.

Introduction Coelenterazine

Coelenterazine is a substrate for bioluminescent enzymes of marine origin like Renilla and Gaussia but also for Aequorin and Obelin.

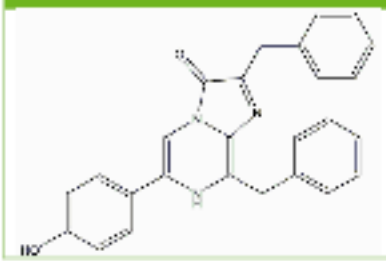
Presently commercially available products have been found to contain less than 85% of the active substance.
However, **both Coelenterazine and deoxy coelenterazine (benzyl-coelenterazine)** supplied by **RESEM** are manufactured using newly developed methods, thus providing extremely pure and active luminophores without the need for extended chromatographic purification procedures.

An aqueous solution of coelenterazine should be freshly prepared, allowed to stabilize 15 minutes at room temperature and used within the same working day.
An alcoholic solution of coelenterazin can be kept in a refrigerator for as long as 3 weeks.
Coelenterazine as yellowish crystals should be stored in a dry and dark place under nitrogen or argon at -80ºC.

Shelf-life for unopened vials is 2 years after manufacturing.

Product Information Coelenterazine

Deoxy Coelenterazine (CLZh)

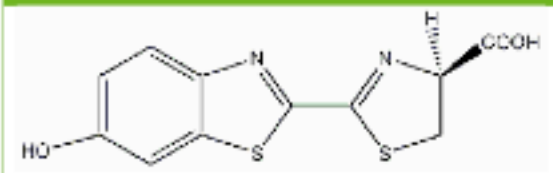


$C_{26}H_{21}N_3O_2$
Mol.Wt: 407.46

Specifications for deoxy Coelenterazine (CLZ h)

- Appearance: powder with intensive yellow colour
- Stocking: at -20 to -80°C in dark and dryness
- Water content: <0.02%
- Chemical purity: >96% by HPLC
- Packed under argon, shipped refrigerated

n Coelenterazine (nCLZ)



$C_{25}H_{25}N_3O_3$
Mol.Wt: 415.48

Specifications for Coelenterazine (nCLZ)

- Appearance: powder with intensive yellow colour
- Stocking: at -20 to -80°C in dark and dryness
- Water content: <0.02%
- Chemical purity: >95% by HPLC
- Packed under argon, shipped refrigerated

Packing information

Packing sizes D-Luciferin & L-Luciferin, - Na-salt ,- K-salt

Packing Sizes	Prod.No.	Purity	Quantity
D-Luciferin, free acid	260159	>99%	1 gram, 5 grams
D-Luciferin Methyl ester	260313	>99%	1 gram
D-Luciferin Sodium salt	260401	>99%	1 gram, 5 grams
D-Luciferin Potassium salt	260402	>99%	1 gram, 5 grams
L-Luciferin, free acid	260201	>99%	1 gram

Packing sizes n Coelenterazine, Coelenterazine h

Packing Sizes	Prod.No.	Quantity
n Coelenterazine	301702	10 mg, 25 mg
Coelenterazine h	301801	10 mg

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