Instructions For Use

Hikarazine™ Z-103, Z108

Synthelis Biotech ©

Biology at speed

Bioluminescent substrates Hikarazine™ Z103 & Z108

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1. Intended use

Hikarazine™ represents a patented[1] class of bioluminescent pro-substrates[2] (commonly referred to as pro-luciferins), specifically designed for a use with luciferase enzymes derived from *Oplophorus gracilirostris*.

Hikarazine™ offers significant advantages, including exceptional long-term storage at room temperature and, upon its hydrolysis into a luciferin, can provide superior brightness and higher sensitivity when aiming at the detection of ultra-low concentration of proteins[3].

Depending on the bioluminescence assay and protocol, 1 mg of **Hikarazine™** can be used for:

- 2,500 to 3,000 bioluminescence immunoassays or enzymatic assays (on lyzed cells for example)
- 750 to 1,500 live cell-based assays

[1] "Imidazopyrazine derivatives, process for preparation thereof and their uses as luciferins", EP3615516, US11938199, JP7366752

[2] "Gram-scale synthesis of luciferins derived from coelenterazine and original insights into their bioluminescence properties", Organic & Biomolecular Chemistry, Coutant et al. (2019)

[3] "A highly sensitive bioluminescent method for measuring allergen-specific IgE in microliter samples", Allergy, Goyard et al. (2020)

- Up to 20 in vivo assays in small animals

Hikarazine™ are Research Use Only (RUO) products and should be used exclusively by personnel trained in research laboratory techniques.

2. Operating principles

Hikarazine™ compounds are pro-luciferins with an O-acetyl group, which ensures excellent stability for shipment or long-term storage at room temperature. These compounds require an activation via hydrolysis, as illustrated in Figure 1 below.

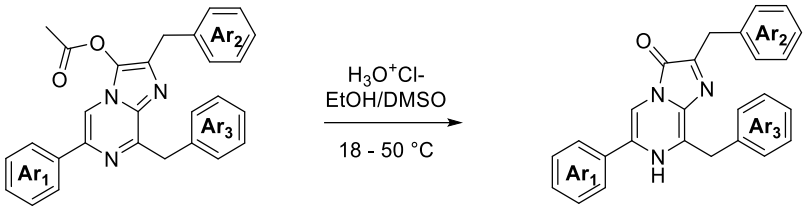

Figure 1 : principle of pro-luciferin activation

Upon hydrolysis, **Hikarazine™ Z108** converts into Q108, a coelenterazine analogue utilized by luciferase enzymes to produce blue light with a peak emission at 470 nm. In many cases, Q108 delivers a 2- to 15-fold enhanced signal compared to standard luciferin, depending on the specific luciferase used. Additionally, it offers a signal half-life of approximately 1 hour.

Upon hydrolysis, **Hikarazine™ Z103** converts into Q103, a coelenterazine analogue leading to a very stable blue signal with a half-life of approximately 3 hours.

As illustrated below for Q108, the bioluminescence reaction catalyzed by the luciferase occurs through the oxidative decarboxylation of coelenterazine, resulting in the release of coelenteramide in an excited state. This excited state then can relax via the emission of a blue photon.

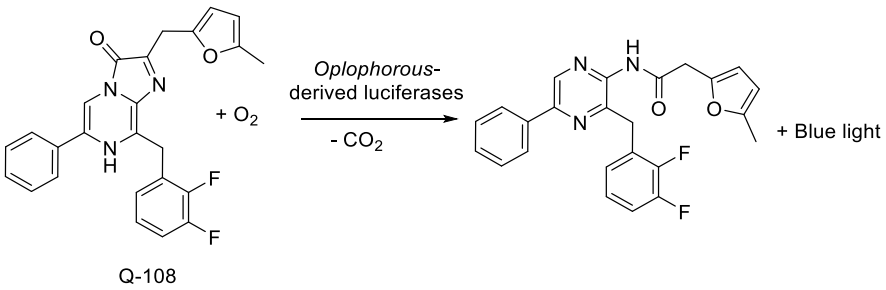

Figure 2: Hikarazine™ Q108 light emission reaction

Note that the brightness produced by this catalytic process is highly dependent on both the luciferase and the buffer used, as these factors do impact the quantum yield of light emission.

3. Materials and methods

Additional Materials and equipment required

- ❒ Ethanol 99%
- ❒ Hydrochloric acid 37%
- ❒ Dimethyl sulfoxide (DMSO)
- ❒ Phosphate Buffered Saline (PBS)
- ❒ Thermomixer or incubator
- ❒ Pipettes and mini-centrifuge
- ❒ Bioluminescence detection system

Step 1: Pro-substrate activation and storage in stock solution

1) Prepare a solution of acidic ethanol by adding 10 µL of 37% hydrochloric acid to 1.2 mL of 99% ethanol.
2) Gently spin down the tube to pellet the **Hikarazine™** at the bottom.
3) To dissolve the pro-luciferin:
4) For **Hikarazine™ Z103**, add 160.2 µL of DMSO.
5) For **Hikarazine™ Z108**, add 155.5 µL of DMSO.
6) To dilute:
7) Add 240.3 µL of acidic ethanol to **Hikarazine™ Z103**.
8) Add 233.3 µL of acidic ethanol to **Hikarazine™ Z108**.
9) Close the vial and incubate the solution at 50°C for 2 hours or at 18°C for 24 hours to produce a stock solution of 5.4 mM **Q103** or **Q108**, the activated luciferin.

Note 1: Substrate activation will result in a colour change from white-yellow to brown.
Note 2: Alternatively, 99% ethanol may be used in place of DMSO.

This concentrated 5.4 mM stock solution can be used immediately after dilution in your chosen buffer. Alternatively, it can be stored at -20°C for at least 6 months, with consistent performance maintained through 5 freeze–thaw cycles.

Table 1: Hikarazine™ features and resuspension volumes

Product	MW	Masse	Final conc	Final vol	Vol. DMSO	Vol. EtOH/Ac
Z103	459.4 g/mol	1 mg	5.4 mM	400.5 µL	**160.2 µL**	**240.3 µL**
Z108	473.5 g/mol	1 mg	5.4 mM	388.8 µL	**155.5 µL**	**233.3 µL**

Step 2: Substrate utilisation

Before utilization, Q103 and Q108 luciferin should be diluted in an appropriate buffer depending on the applications.
The diluted substrate should be used during the day and preferentially kept at 4°C. At room temperature, an oxidation process will result in a rather fast loss of signal over time.
Hikarazine™ substrates can be used for a variety of applications, including immunoassays, cell-assays or *in vivo* assays:

- ❒ For immunoassays in 96 well-plates, it is recommended to use 50 µL of Q103 or Q108 per well at a final concentration of 13.6 µM (400 times dilution of the stock solution).
- ❒ For live cell assays, it is recommended to use Q103 or Q108, at a final concentration of 27 µM per assay (200 times dilution of the stock solution). Typically, 100µl of substrate at 54 µM (100 times dilution of the stock solution) is added to 100µl of culture medium for 96 well-plates.
- ❒ For in vivo testing in small animals[4], intraperitoneal injection of 2 to 4 mg/kg is recommended. Typically, that represents 10 to 25 µl of Q-103 or Q-108 stock solution dilute in 100 µL of PBS with a final concentration 0.4 mM to 1 mM. The substrate should preferably be injected shortly after dilution.

The light emission should be measured using a luminometer, a plate reader or imaging system capable of detecting light at 470 nm. The intensity of the emitted light is measured in Relative Light Units (RLU), with higher RLU values indicating higher luciferase activity and thus higher concentrations of the target protein.

For accurate and reliable measurements, ensure the following:

- ❒ Immediate measurement after substrate addition to capture the peak light emission.
- ❒ Use of appropriate controls, including a blank to subtract background noise and a positive control to verify the efficiency of the luciferase system.

4. Storage conditions and stability

The pro-luciferins Hikarazine™ Z103 and **Hikarazine™ Z108** are stable for up to 2 years at room temperature (+15°C to +25°C) in a sunlight free and dry environment.
Once activated, the concentrated and acidic stock solutions of **the resulting luciferins Q103** or **Q108** can be stored for at least 6 months at -20°C.

5. Product content

Table 2: Catalogue numbers of Hikarazine products

Product	Size	Reference
Hikarazine™ Z103 1mg	1 x 1mg	SYN90006-1-01
Hikarazine™ Z103 5mg	5 x 1mg	SYN90006-5-01
Hikarazine™ Z103 10mg	10 x 1mg	SYN90006-10-01
Hikarazine™ Z108 1mg	1 x 1mg	SYN90007-1-01

[4] Belarbi, E.; Legros, V.; Basset, J.; Després, P.; Roques, P.; Choumet, V. Bioluminescent Ross River Virus Allows Live Monitoring of Acute and Long-Term Alphaviral Infection by In Vivo Imaging. *Viruses* **2019**, *11*, 584. https://doi.org/10.3390/v11070584

Hikarazine™ Z108 5mg	5 x 1mg	SYN90007-5-01
Hikarazine™ Z108 10mg	10 x 1mg	SYN90007-10-01

6. Safety information

To be handled only by personnel trained in research laboratory techniques. Suitable protective laboratory equipment (lab coat, disposable gloves, and safety glasses) should be worn when handling **Hikarazine™**.

Hikarazine™ may be harmful if swallowed, may cause skin irritation, may cause serious eye irritation, may cause respiratory irritation.

Disposal should be in accordance with applicable regional, national and local laws regulations.

7. Legal

Researchers may use this product for research use only. No commercial use is allowed. Commercial use means any and all uses of this product by a party in exchange for consideration, including, but not limited to (i) use for further product manufacture, (ii) use in provision of services, (iii) resale of product. No other use or transfer of this product is authorized without the prior express consent of Synthelis Biotech.

8. List of symbols used


Manufacturer's name and address

For research Use Only


Temperature limits between which the product can be safely exposed


Product Sensitive to humidity


Consult the user guide

See accompanying documents