

Illumination™ Renilla Luciferase Enhanced Assay Procedure for Luciferase Reporter Assay

Introduction

Renilla luciferase is a commonly used bioluminescent reporter gene that is used to study gene regulation and function both *in vivo* as well as *in vitro*. *Renilla* luciferase is a monomeric 36 kDa protein isolated from the sea pansy, *Renilla reniformis*, which catalyzes coelenterazine with oxygen to produce a blue light (480 nm). There is no need for any post-translational modifications and the protein may be used as a gene reporter immediately after translation. Our Enhanced Assay Kit includes a special buffer which is designed to yield reliable, linear measurements of *Renilla* luciferase activity with maximum sensitivity and minimal autoluminescence. This kit is a flash-type luminescent assay, and requires measurement immediately after adding the substrate to the sample.

GoldBio's Illumination™ *Renilla* Luciferase Enhanced Assay Kit is specifically designed for the measurement of *Renilla* luciferase in transfected cell reporter gene assays. This kit is enhanced with a water soluble coelenterazine that is easier to use and store than other conventional coelenterazine products. The enhanced coelenterazine can be stored at -20°C with minimal evaporation, unlike methanol solutions of coelenterazine. This kit is a flash-type luminescent assay, and requires measurement immediately after adding the substrate to the sample. The luminescence signal decreases about 50% after about 3-4 minutes of reaction time (Figure 2), although signal half-life may vary depending on luciferase expression levels.

Materials

Table 1. Kit Components

Component	I-925-50 (50 assays)	I-925-150 (150 assays)	I-925-1000 (1000 assays)
5X Passive Lysis Buffer	5 ml (Catalog # L-745)	10 ml (Catalog # L-745)	30 ml (Catalog # L-745)
<i>Renilla</i> Luciferase Assay Buffer	5 ml 50 assays	15 ml 150 assays	100 ml 1000 assays
GoldBio Enhanced Coelenterazine	1 x 200 µg	3 x 200 µg	1 x 4 mg

Note: Sufficient lysis buffer is provided to perform the stated number of assays with cells grown from 96 well to 24 well plates. For applications requiring more lysis buffer (e.g. >100 µl/well), additional 5X Passive Lysis Buffer ([Catalog # L-745](#)) may be purchased separately.

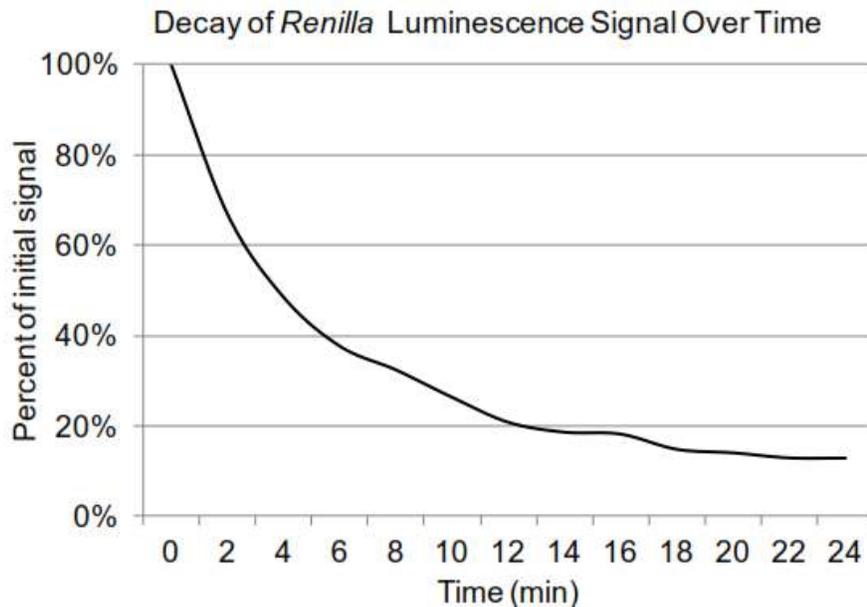
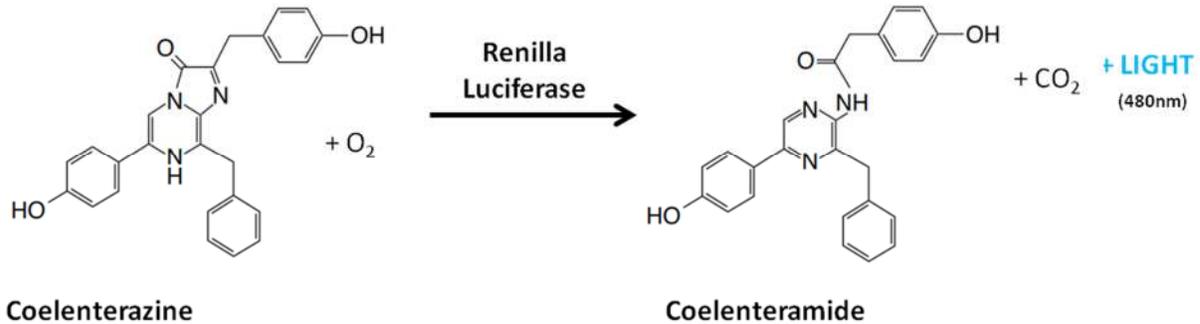


Figure 2. Stability of luminescence signals in the *Renilla* Luciferase Assay 2.0. Luminescence measurements were carried out in a white 96-well plate on cells transfected with a plasmid expressing *Renilla* luciferase. Luminescence was measured using a Bio-Tek H1m microplate reader every 2 minutes for 24 minutes, and RLU values were normalized to the first measurement for each reaction.

Storage/Handling

Store the kit at $-80^{\circ}C$. *Renilla* Luciferase Assay Buffer is stable at $-80^{\circ}C$ for three months. The other kit components are stable at $-20^{\circ}C$ for at least six months from date of receipt. Kit components and enhanced coelenterazine stock solutions in water are stable to at least 5 freeze-thaw cycles.

Method

Preparation of Cell Lysates

1. Preparation of Passive Lysis Buffer
 - a. Prepare 1X passive lysis buffer by adding 1 volume of 5X passive lysis buffer to 4 volumes of dH₂O and mixing well. 1X lysis buffer may be stored at 4°C for up to one month. Store 5X passive lysis buffer at -20°C.
2. Lysis of Cells Cultured in Multiwell Plates
 - a. Remove growth medium from cultured cells and gently add a sufficient volume of phosphate buffered saline (PBS) ([GoldBio Catalog # P-271](#)) to wash the surface of the culture vessel. Remove the PBS and add 1X passive lysis buffer to each well using the volume recommended below for each type of culture plate:

Wells/plate	Lysis buffer/well
6 well	500 µl
12 well	250 µl
24 well	100 µl
48 well	65 µl
96 well	20 µl

- b. Place the culture plates on a rocking platform or orbital shaker with gentle rocking/shaking to ensure complete and even coverage of the cell monolayer with 1X passive lysis buffer. Rock the culture plates at room temperature for 15 minutes.

Note: Cultures that are overgrown are often more resistant to complete lysis and typically require an increased volume of passive lysis buffer and/or an extended treatment period to ensure complete lysis. Lifting cells from the plate will facilitate the process of cell lysis. See GoldBio's [Luciferin In Vitro Handbook](#) for more tips and suggestions.

- c. Transfer the lysate to a tube or vial. Place at 4°C until ready to assay. Store lysates at -20°C or -80°C if assay will not be performed on the same day.

Note: (Optional). The lysate can be cleared by centrifugation for 30 seconds at top speed in a refrigerated microcentrifuge and transferred into a new tube.

Preparation of *Renilla* Working Solution

1. Thaw *Renilla* Luciferase Assay Buffer at room temperature.
2. Prepare 2 mg/ml enhanced coelenterazine stock solution. For **I-925-50** or **I-925-150**, add 100 µl dH₂O to the vial (200 µg) and mix. For **I-925-1000**, add 2 ml dH₂O to the vial (4

mg) and mix. The stock solution can be stored for at least 3 months at -20°C or below, and is stable to up to 5 freeze/thaw cycles.

3. Prepare enough *Renilla* working solution to perform the desired number of assays (100 µl working solution per assay). Add enhanced coelenterazine (2 mg/ml) to *Renilla* luciferase assay buffer at a ratio of 1:50. For example, add 20 µl enhanced coelenterazine stock solution to 1 ml assay buffer.

Note: For best results, working solutions (assay buffer with substrate) should be prepared fresh before each use, and used within 3 hours of preparation. *Renilla* working solution activity is stable up to 3 hours, but background increases up to 60% after 5 hours at room temperature.

Renilla Luciferase Assay

1. For manual luminometer:
 - a. Set up luminometer with appropriate parameters (delay time, integration time, sensitivity, etc.).
 - b. Add 100 µl of *Renilla* luciferase working solution to the luminometer tube.
 - c. Add 20 µl of cell lysate and mix quickly by vortexing or flicking the tube with a finger.
 - d. Place tube in luminometer and initiate measurement. Luminescence is normally integrated over 10 seconds without delay. Other integration times may also be used.
 - e. If the luminometer is not connected to a printer or computer, record the *Renilla* luciferase activity measurement.
 - f. Discard the reaction tube, and proceed to the next *Renilla* luciferase reaction.
2. For luminometer with injector:
 - a. Format the luminometer so that the injector dispenses 100 µl. Prime the injector with *Renilla* luciferase working solution.
 - b. For each reaction, carefully add 20 µl of cell lysate to an individual luminometer tube or to the wells of a multiwell plate.
 - c. Place the samples in a luminometer.

- d. Initiate measurement. This will cause *Renilla* luciferase working solution to be injected into the reaction vessel and the measurement to be subsequently taken. Luminescence is normally integrated over 10 seconds without delay. Other integration times also may be used.
- e. Record the *Renilla* luciferase activity measurement.
- f. If using a single tube luminometer, discard the reaction tube, and proceed to the next *Renilla* luciferase reaction. If using a plate luminometer, the luminometer will automatically begin injecting *Renilla* luciferase working solution into the next well indicated on the luminometer plate.

Determination of Assay Background

The expression of a luciferase reporter is quantified by the luminescence produced above background levels. In most cases, background created by the reagent in the absence of luciferase is very low compared to signal with luciferase. However, when measuring low levels of luciferase activity, it is important to subtract the background signal from untransfected cells or cells transfected with a negative control vector from measurements of luciferase activity.

Associated Products

GoldBio Catalog #	Product Name
I-930	Illumination™ Firefly Luciferase Enhanced Assay Kit
I-920	Illumination™ Firefly & <i>Renilla</i> Luciferase Enhanced Assay Kit
LUCK	D-Luciferin, Potassium Salt (Proven and Published™)
LUCNA	D-Luciferin, Sodium Salt (Proven and Published™)
CA	Coelenterazine
L-745	5X Passive Lysis Buffer

Materials from GoldBio are sold for research use only, and are not intended for food, drug, household, or cosmetic use.