Illumination™ Dura-Luc Lyophilized Firefly HTS Assay

Procedure for Luciferase Reporter Assay

Introduction

Firefly Luciferase Assays are one of the leading reporter assays in the world in the measurement of gene function and gene regulation as well as being widely used in pharmaceutical screening. The luciferase assays are sensitive and convenient due to the absence of endogenous luciferase activity in most cell types and tissue. Firefly luciferase is a monomeric 62 kDa protein typically isolated from the firefly, *Photinus pyralis*, which catalyzes the ATP-dependent D-luciferin in the presence of oxygen and Mg^{2+} to oxyluciferin producing a yellow to greenish light (~560 nm). However, the light production resulting from the reaction leads to formation of suicidal adenyl-oxyluciferin at the enzyme surface. It results in very short half-life of the light emission with a flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface, but the signal duration (10-15 minutes) is still too short for batch process screening.

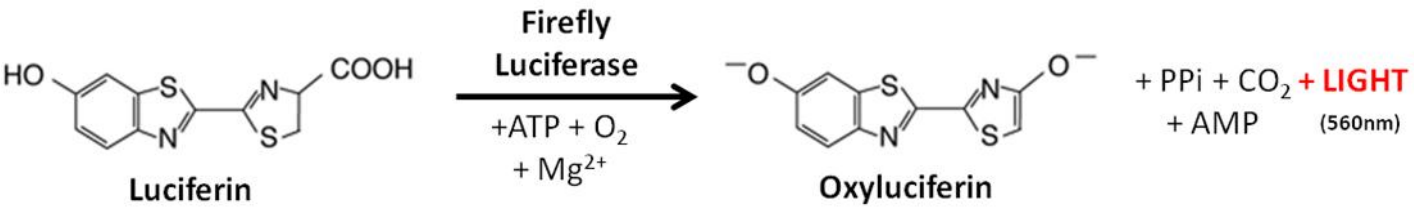
GoldBio's Illumination™ Dura-Luc HTS assay system is a proprietary mixture of substances that modify the enzymatic reaction to produce a long lasting signal (steady glow) by preventing the formation of adenyl-oxyluciferin at the enzyme surface. It is a homogeneous high sensitivity firefly luciferase reporter gene assay kit for the quantification of firefly luciferase expression in mammalian cells with signal half-life of about 3 hours. Glow-type luciferase assays like Steady-Luc have lower luminescence signal compared to flash-type assays. The sensitivity and limit of detection of the assay will depend on luciferase expression levels in your experimental system as well as luminometer sensitivity.

Kit Components

Component	I-946-120 (120 assays)	I-946-1000 (1000 assays)	I-946-10000 (10,000 assays)
Dura-Luc Reconstitution Buffer	12 ml	100 ml	10 x 100 ml
Dura-Luc Assay Buffer (Lyophilized)	1 bottle	1 bottle	10 bottles
GoldBio D-Luciferin	3 x 1 mg (Catalog # LUCK/LUCNA)	25 mg (Catalog # LUCK/LUCNA)	10 x 25 mg (Catalog # LUCK/LUCNA)

Gold Biotechnology
St. Louis, MO
Ph: (314)890-8778
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

=



Storage/Handling

Store the kit at -20°C. The kit is stable at -20°C for at least six months from date of receipt. After reconstitution, aliquot Dura-Luc Assay Buffer, if necessary, to avoid repeated freeze-thaw cycles; reconstituted Dura-Luc Assay Buffer is stable at -80°C for at least 6 months.

Method

Note: Dura-Luc luminescence signal has a half-life of about 3 hours, but may fluctuate over time or with temperature variation, and may vary depending on culture medium used. Therefore, raw luminescence values should be directly compared only for samples in the same medium. For comparison of luminescence signal between plates that are read at different times, each plate should include the same common internal control. The luminescence signals from each plate can be normalized to the internal control from the same plate.

Note: Dura -Luc assay should be carried out on cells or samples in cell culture medium containing magnesium. Luminescence signal will be low in the absence of magnesium.

1. Equilibrate the kit components to room temperature.

2. Prepare the lyophilized Dura-Luc assay buffer by adding reconstitution buffer to the bottle containing the lyophilized buffer. For I-946-120, add 12 ml reconstitution buffer, for I-946-1000 and I-946-10000, add 100 ml of reconstitution buffer per bottle. Mix by rocking until the buffer is a homogenous solution. Reconstitution buffer contains detergent; mix gently to avoid excessive foaming. Reconstituted assay buffer is stable at -20°C for at least 3 months or -80°C for at least 6 months. Avoid repeated freeze-thaw cycles.

3. To prepare Dura-Luc working solution, mix D-luciferin substrate and Dura-Luc assay buffer in 1 mg to 4 ml ratio (i.e., for each 1 mg vial of D-luciferin, mix with 4 ml assay buffer or for each 25 mg vial of D-luciferin, mix with 100 ml assay buffer). Add a small volume of assay buffer to the D-luciferin vial and mix by inversion until the substrate is completely dissolved, then transfer the D-luciferin solution to the full volume of assay buffer required. Only prepare working solution as needed for one day.

   Note: D-luciferin in assay buffer has limited stability. Instead of dissolving the entire contents of the D-luciferin vial in assay buffer, you may prepare a D-luciferin stock solution at 10 mg/ml in dH_2O, and store it at -20°C or below for repeated use. The D-

=

luciferin stock solution should be stable for at least one month, depending on the frequency of freeze-thaw cycles. Aliquots may also be made to reduce freeze-thaw cycles. The required volume of working solution can be prepared by diluting D-luciferin in assay buffer to a final concentration of 0.25 mg/ml (2.5 µl of 10 mg/ml D-luciferin stock solution per 100 µl assay buffer).

4. Remove plates containing luciferase-expressing cells from the incubator. If plates will be read in luminescence microplate reader, make sure plates are compatible with the instrument.

5. Add a volume of assay solution equal to that of the culture medium in each well and mix well. For example, for 96-well plates, add 100 µl assay solution to each well containing 100 µl of cells in medium, for a final volume of 200 µl per well.
   Note: If the culture media does not contain magnesium, add 10mM $MgSO_4$ to the assay solution.

6. Wait at least 5 minutes for complete lysis of the cells. Mixing on an orbital shaker during cell lysis is recommended.

7. Immediately before reading luminescence, mix samples thoroughly. Measure luminescence with a microplate luminometer. Alternatively, cell lysates can be transferred to tubes to be measured in a single sample luminometer. Dura-Luc firefly luciferase expression in mammalian cells has a signal half-life of about 3 hours.
   Note: Luminescence may fluctuate over time or with temperature variation, and may vary depending on culture medium used. For comparison of luminescence signal between plates that are read at different times, each plate should include the same common internal control.

Related Products

GoldBio Catalog #	Product Name
I-930	Illumination™ Firefly Luciferase Enhanced Assay Kit
I-940	Illumination™ Firefly Luciferase Stabilizer
LUCK	D-Luciferin, Potassium Salt (Proven and Published™)
LUCNA	D-Luciferin, Sodium Salt (Proven and Published™)
I-920	Illumination™ Firefly & Renilla Luciferase Enhanced Assay Kit