Revised: March 13, 2023

Product Information

Steady-Luc™ Firefly HTS Assay Kit

Kit Contents:

Component	30028-T 40 assays	30028-1 120 assays	30028-2 1,000 assays	30028-3 10,000 assays
D-Luciferin	99907 1 mg	99907 3 x 1 mg	30028A2 25 mg	30028A2 10 x 25 mg
Steady-Luc™ Assay Buffer	30028B-T 4 mL	30028B 12 mL	30028B2 100 mL	30028B2 10 x 100 mL

Steady-Luc™ Firefly HTS Assay Kit (Lyophilized)

Kit Contents:

Component	30028-L1 120 assays	30028-L2 1,000 assays	30028-L3 10,000 assays
D-Luciferin	99907 3 x 1 mg	30028A2 25 mg	30028A2 10 x 25 mg
Steady-Luc™ Assay Buffer (Lyophilized)	30028L-B1 1 bottle	30028L-B2 1 bottle	30028L-B2 10 bottles
Steady-Luc™ Reconstitution Buffer	30028L-C1 12 mL	30028L-C2 100 mL	30028L-C2 10 x 100 mL

Number of assays is based on 96-well plate format.

Storage and Handling

Store Steady-Luc™ Firefly HTS Assay Kit (30028 series) at <-60°C. Kit components are stable for at least six months from date of receipt when stored as recommended. Avoid repeated freeze-thaw cycles.

Store Steady-Luc™ Firefly HTS Assay Kit (Lyophilized) (30028-L series) at –20°C. Kit components are stable for at least six months from date of receipt when stored as recommended. See protocol on page 2 for reconstitution instructions.

Product Description

Firefly luciferase is widely used as a reporter for studying gene regulation and function, and for pharmaceutical screening (1, 2). It is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells or tissues (3, 4). Firefly luciferase is a 62,000 Dalton protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-Luciferin oxidation by oxygen into oxyluciferin with emission of light centered at 560 nm (Figure 1).

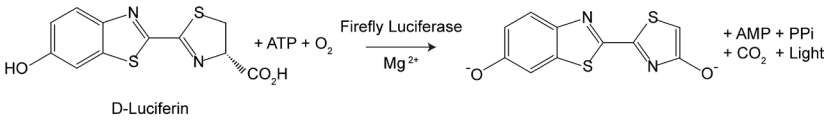
However, the light production resulting from the reaction leads to formation of spontaneously decaying adenyl-oxyluciferin at the enzyme surface. The result is a light emission with a very short half-life with flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface (5, 6), but the signal duration (10-15 minutes) is still too short for batch process screening.

Biotium's Steady-Luc™ HTS assay system is a homogeneous high-sensitivity firefly luciferase reporter gene assay for quantifying firefly luciferase expression in mammalian cells with a signal half-life of about 3 hours (Figure 2). The kit contains a proprietary mixture of substances that modify the enzymatic reaction to produce a long-lasting signal (steady glow) by preventing adenyl-oxyluciferin formation at the enzyme surface. 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.

Biotium's original Steady-Luc™ Firefly HTS Assay Kit (30028 series) contains assay buffer in liquid format. Steady-Luc™ Firefly HTS Assay Kit (Lyophilized) (30028-L series) is a newer packaging format that includes lyophilized assay buffer for convenient room temperature shipping and storage at -20°C.

References

1. Anal Biochem 188, 2 (1990); 2. Anal Biochem 219, 2 (1994); 3. Anal Biochem 175, 1 (1988); 4. BioTechniques 7, 10 (1989); 5. Proceedings of the VIth International Symposium on Bioluminescence and Chemiluminescence, Cambridge, (1990); 6. Biochemica and Biophysica Acta 27 (1958)



D-Luciferin

Figure 1. Bioluminescent reaction catalyzed by firefly luciferase.

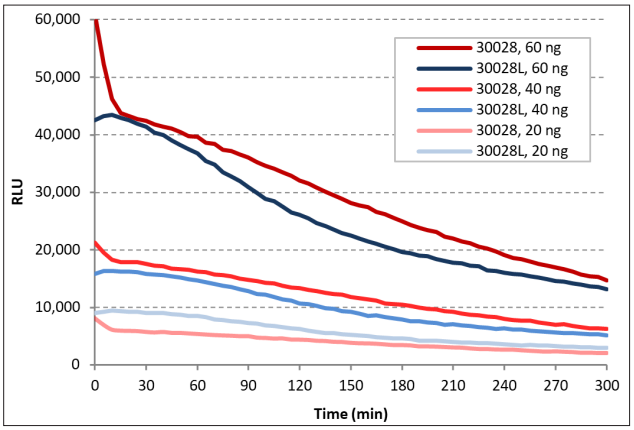


Figure 2. Steady-Luc™ (30028) and Steady-Luc™ (Lyophilized) (30028-L) assays in transfected CHO-K1 cells. CHO-K1 cells were grown in F12-K medium containing 10% FBS in a white 96-well plate. On the day after plating, cells were transiently transfected with varying amounts of pGL3 firefly luciferase expression vector (Promega) using ViaFect™ transfection reagent (Promega). On the day after transfection, the medium was replaced with fresh growth medium (100 uL per well). The plate was allowed to equilibrate to room temperature, and 100 uL Steady-Luc™ assay buffer or reconstituted Steady-Luc™ Assay Buffer (Lyophilized) containing D-Luciferin was added to each well. The plate was placed in a BioTek® Synergy H1 microplate reader and mixed with fast orbital shaking for five minutes. Luminescence was read every five minutes for five hours, with three seconds of orbital shaking before each read. Background luminescence values from untransfected cells were subtracted from luminescence values for each time point. Averaged luminescence values for duplicate wells are shown for various amounts of pGL transfected (ng/per well).

Assay Procedure

Note: Steady-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: Steady-Luc™ assay should be carried out on cells or samples in cell culture medium containing magnesium. Luminescence signal will be low otherwise.

Protocol

1. Equilibrate the kit components to room temperature (22°C).
2. For lyophilized format (30028-L series) only:
   a. Thaw the Reconstitution Buffer (Component C) and bring to room temperature. Swirl gently to mix. If a gel is observed at the bottom of the bottle, warm to 37°C and swirl or rock the bottle gently until the Reconstitution Buffer is fully dissolved.

      Note: To avoid foaming, do not shake or vortex the bottle.

   b. Add Reconstitution Buffer (Component C) to the Lyophilized Assay Buffer (Component B) as described below:

      - For 30028L-B1, add 12 mL of Reconstitution Buffer to the bottle containing Lyophilized Assay Buffer.
      - For 30028L-B2, add 100 mL of Reconstitution Buffer to the bottle containing Lyophilized Assay Buffer

   c. Swirl or rock the bottle of Assay Buffer gently to dissolve.

      Note: To avoid foaming, do not shake or vortex the bottle.
3. To prepare Steady-Luc™ working solution, mix D-Luciferin substrate and Steady-Luc™ Assay Buffer in a ratio of 1 mg to 4 mL (i.e., mix the vial of 1 mg D-Luciferin with 4 mL Assay Buffer, or mix the 25 mg vial of D-Luciferin 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. 10 mg/mL D-Luciferin in water is stable for at least 1 year and at least 5 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 uL of 10 mg/mL D-Luciferin stock solution per 100 uL assay buffer).
4. Remove plates containing luciferase-expressing cells from the incubator. If the plates will be read in a luminescence microplate reader, make sure they are compatible with the instrument.
5. Add a volume of assay solution equal to that of the culture medium in each well and mix thoroughly. For example, for 96-well plates, add 100 uL assay solution to each well containing 100 uL of cells in medium, for a final volume of 200 uL per well.
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.

Related Products

Cat. No.	Product
10100	D-Luciferin, Free Acid
10101	D-Luciferin, Potassium Salt
10102	D-Luciferin, Sodium Salt
10110 - 10126	Coelenterazine Analogs
30005	Firefly & Renilla Dual Luciferase Assay Kit
30075	Firefly Luciferase Assay Kit (Lyophilized)
30020	ATP-Glo Bioluminometric Cell Viability Assay
30081	Firefly & Renilla Luciferase Single Tube Assay Kit
30082	Renilla Luciferase Assay Kit 2.0
30085	Firefly Luciferase Assay Kit 2.0
22003	Mini Cell Scrapers, pack of 200

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