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Dual Luciferase Assay Reagent for Renilla and Red Firefly Luciferases

Catalog no.	Size	Description	Price
DLAR-2	1000 assays	Renilla Luciferase-Red-emitting Firefly (Luciola) Luciferase Dual Assay Reagent	$850.00

Description

DLAR-2: Single Solution-based Dual Luciferase Assay Reagent

Save on cost and time in screening applications

A panel of improved, ultra-sensitive secreted luciferase reporters have been developed in an effort to enable analysis of different promoter activities in the same group of transfected cells. This approach not only enables analysis of three or more pathways (responses) in the same group of cells but also enables one to study the response in real time without killing cells (since the three reporters are secreted). By choosing luciferases with different emission maxima we provide an additional advantage: multiple luciferases can be assayed using a single assay reagent and the luciferase activities can be spectrally resolved using appropriate filters. The single-solution based DLAR-2 dual assay reagent is based on the luciferase reporters listed below:

Green-emitting Renilla Luciferase: The emission max of the Green-emitting Renilla luciferase (530 nm) makes it ideal for multiplexed assays with blue and red emitting luciferases. This luciferase has been engineered for improved brightness (about 40 times brighter than human codon optimized native Renilla Reniformis Luciferase) and extended stability of the bioluminescent signal.

A Red-emitting luciferase from the Italian firefly Luciola Italica: The emission wavelength of the red Luciola luciferase is 617 nm. The robust signal of our Luciola luciferase mutants, which have a 1000 times higher signal intensity compared to native Luicola luciferase, makes them attractive for single solution-based multiplexed assays, as signal strength is typically diminished in single solution-based multiplexed assays wherein different luciferase activities are spectrally resolved using appropriate filters.

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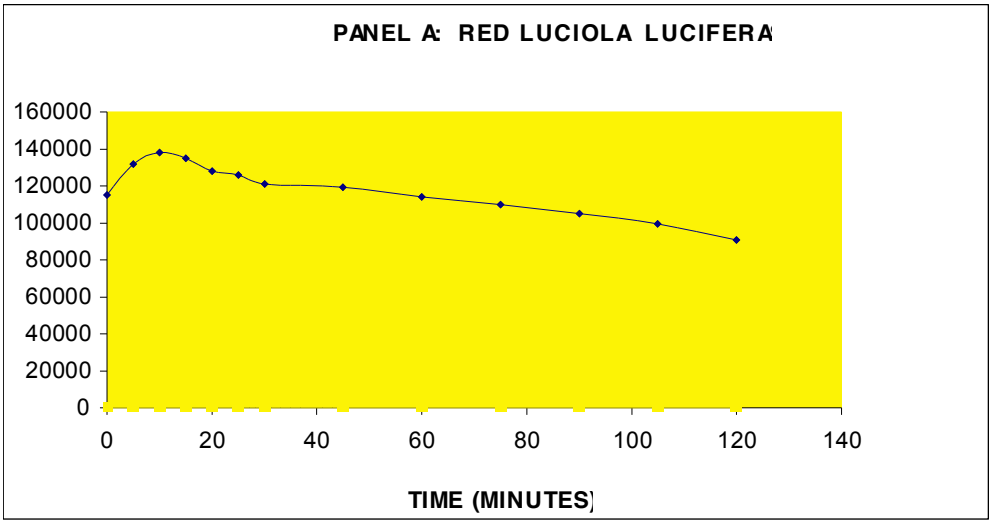
Figure 1: Stability of the bioluminescent signal of Firefly luciferase (Panel A) and Green Renilla Luciferase (Panel D) using the DLAR-2 reagent.
This reagent is useful for HTS applications involving both Renilla Luciferase and the red-emitting Luciola Luciferase.
Note: Data is an average of triplicate determinations measured on a Turner TD2020 luminometer.



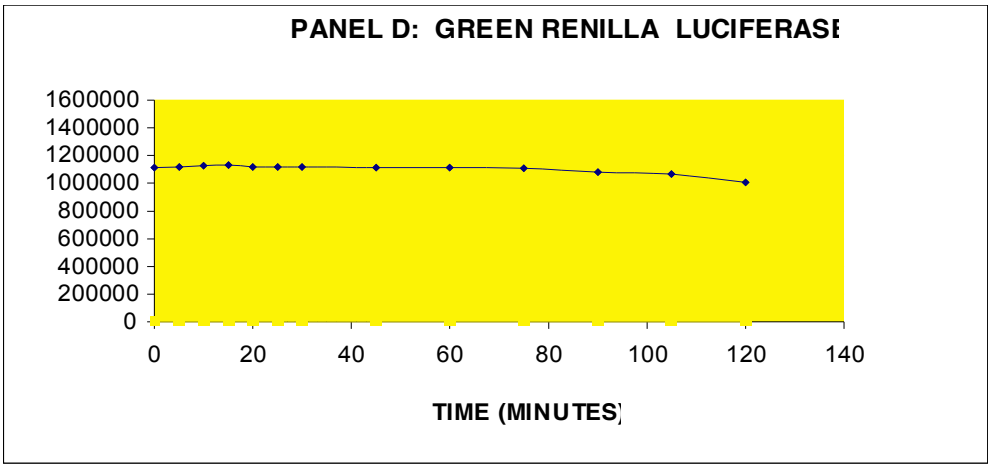


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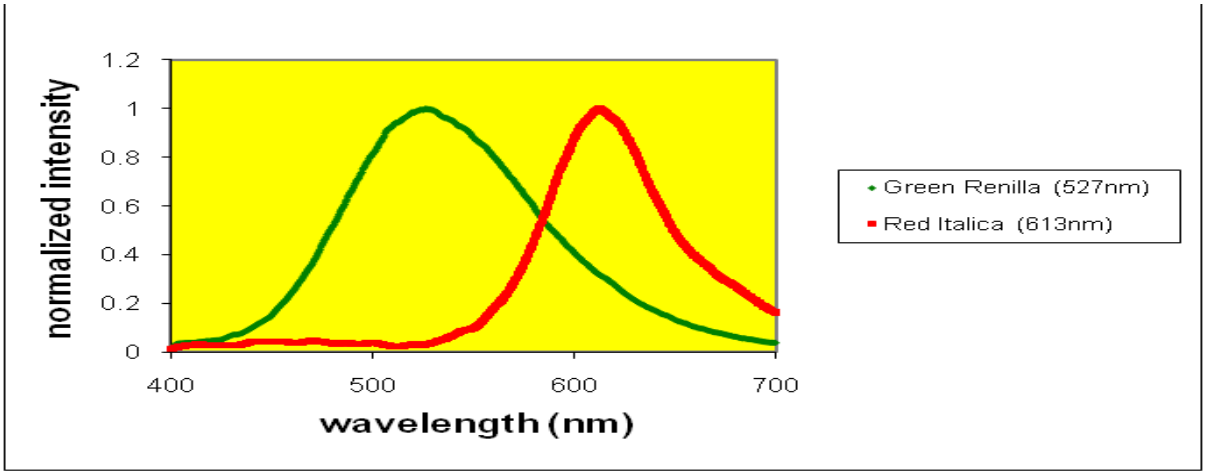
Figure 2: Emission spectra of Green Renilla Luciferase (green) and Red Firefly Luciferase (red) in samples of transfected cells (lysates or supernatants).
The emission spectra were recorded on a Fluorolog-3 spectrofluorometer (Horiba Scientific, Japan) using a liquid nitrogen cooled CCD. The luciferases were assayed by mixing 200 ul of the sample with the appropriate luciferase assay reagent to obtain spectral profiles. Emission max of Green-emitting Renilla Luciferase is 527 nm; Red Italica is 617 nm.
Data courtesy of Justin Rosenberg, Dr Bruce Branchini´s lab, Connecticut College, USA.



Advantages

♦ The improved secreted Green Renilla Luciferase as well as the Red-emitting Luciola Luciferase have very robust signals in both supernatants and lysates of transfected cells. Therefore, they can be used as a dual secreted reporters or dual intracellular reporters.

♦ The stabilizer component of this assay system provides steady kinetics for the Renilla bioluminescent signal over a longer time period, giving users the time required for high-throughput analysis as well as manually delivered assays.

♦ The emission spectra of Green-emitting Renilla Luciferase and the Red-emitting Luciola Luciferase show very little overlap and therefore can be easily resolved spectrally using appropriate filters (3).

♦ The samples containing both Renilla Luciferase and Red firefly luciferase (i.e. growth media or cell lysates after transfection) can be kept at -20°C for long-term storage (5).

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Assay Protocol for DLAR-2 (Gaussia-Red Luciola luciferase dual assay reagent)

Kit Contents

1. DLAR 2 (Renilla-Firefly luciferase Dual Assay Buffer, 100 ml), (Store at -20°C)
2. Renilla luciferase stabilizer (RLAR stabilizer), (Store at -4°C)
3. 100x Coelenterazine (Store at -20°C)
4. 5X-CLR-1 (Store at - 4°C)

Protocol

1. Dilute 10 µl of 100X Renilla luciferase substrate (coelenterazine) per 1 ml using DLAR-2 assay buffer.
2. Mix well by inverting several times.
3. Add 8 ul of RLAR stabilizer to 10 or 20 ul of sample (this is optional and recommended only for HTS applications).
4. Add 100 ul of DLAR -2 reagent, mix well, and read in luminometer.

Note: For reading in a plate luminometer pipette samples into wells first. Then add assay reagents as described above.

Intracellular luciferase activity

Lyse cells using our lysis buffer (Catalog no. 5X CLR-01). Follow cell lysis protocol supplied with the product. Assay as above using 5 ul to 10 ul of lysate.

Note: If you need to measure intracellular luciferase activity, lyse cells first using the cell-lysis buffer from Targeting Systems (catalog no. 5X CLR-01).

1. Dilute the 5X CLR buffer 1:5 with water.
2. Aspirate cell culture media and wash cells twice with serum free DMEM.
3. Add enough of 1X cell lysis buffer to cover cells. Add enough lysis buffer to cover cell.s (50 ul for 96-well, 300 ul for a 12-well, 800 ul for a 6-well dish, and 3 ml for a 10 cm dish).
4. Shake for 20 min at 400 rpm on an orbital shaker (room temperature).
5. Mix 5-20 µl of luciferase-containing sample or cell lysate with 8 ul of RLAR stabilizer (optional) and 100 µl of the luciferase assay reagent (DLAR-2). Read immediately in luminometer.

All assay reagents should be close to room temperature at the time of assay.

Filter Selection: We recommend a 620 nm long pass filter to read the Red-emitting Firefly Luciferase and a 530 nm short pass filter to read the Renilla Luciferase.

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Custom Reagents

We can provide custom formulations to fit your HTS application.

Call our tech support team at 1-866-620-4018, or email us at info@targetingsystems.com or targetingsystems@gmail.com.

Please check our website www.targetingsystems.net for novel luciferase-based multiplexed assays.

References

1) Elisa Michelini, Luca Cevenini, Laura Mezzanotte, Danielle Ablamsky, Tara Southworth, Bruce Branchini, and Aldo Roda* (2007) Spectral-Resolved Gene Technology for Multiplexed Bioluminescence and High-Content Screening. *Anal. Chem.,* 10.1021/ac7016579 S0003-2700(70)01657-8
2) BR Branchini, TL Southworth, JP DeAngelis, A Roda, and E Michelini (2006) Luciferase from the Italian firefly Luciola italica: molecular cloning and expression. Comp Biochem Physiol B Biochem Mol Biol, Oct 2006; 145(2): 159-67.
3) BR Branchini, DM Ablamsky, MH Murtiashaw, L Uzasci, H Fraga, and TL Southworth (2007) Thermostable red and green light-producing firefly luciferase mutants for bioluminescent reporter applications. Anal Biochem, Feb 2007: 361(2): 253-62.

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INFORMATION ON RELATED PRODUCTS

LUCIFERASE ASSAY REAGENTS

Catalog no.	Size	Description	Price
SINGLE LUCIFERASE ASSAYS			
GAR-1	1000 assays	Gaussia Luciferase Assay	$375
GAR-2 0090A	1000 assays	Gaussia Luciferase Assay (Stable signal)	$400
GAR-2B	1000 assays	Gaussia Luciferase Assay (Brighter, Stable version)	$420
RLAR-1	1000 assays	Renilla Luciferase Assay reagent	$400
VLAR-1	1000 assays	Cypridina (Vargula) Luciferase Assay Reagent	$400
VLAR-2	1000 assays	Cypridina Luciferase Assay reagent (more stable version)	$440
FLAR-1	1000 assays	Firefly Luciferase Assay Reagent	$350
DUAL LUCIFERASE ASSAYS			
DLAR-1	1000 assays	Gaussia-Red Firefly Luciferase	$850
DLAR-2	1000 assays	Renilla-Red Firefly Luciferase	$850
DLAR-3	1000 assays	Cypridina-Red Firefly Luciferase	$850
DLAR-4	1000 assays	Cypridina-Gaussia Luciferase	$850
DLAR-5	1000 assays	Cypridina-Renilla Luciferase	$900
TRIPLE LUCIFERASE ASSAYS			
TLAR-1	1000 assays	Cypridina-Green Renilla-Red Firefly Luciferase Assay Reagent	$1000
TLAR-2	1000 assays	Cypridina Luciferase Gaussia Luciferase, red Firefly Luciferase Assay reagent	$1000