

## QUICK PROTOCOL:

### DLAR-2B Firefly -Renilla Dual Luciferase Assay Reagent

#### Catalog # DLAR-2B

*An ultrasensitive dual luciferase reporter assay for sequential measurement of Firefly luciferase and Renilla luciferase in the same sample.*

*Saves costs and time in screening applications*

### Catalog #DLAR-2/DLAR-2B Renilla-Firefly Luciferase Dual Assay Kit

COMPONENT	DESCRIPTION
1.DLAR-2B Component 1	Firefly luciferase assay reagent
2.DLAR-2B Component 2	Renilla luciferase assay buffer (PLUS STOP)
3. 5X CLR (Optional reagent)	5X Cell lysis buffer (not needed when assaying 96-well or 384-well dishes)
4. 100X Renilla Substrate	Coenterazine substrate for assaying Renilla luciferase activity

#### STORAGE TEMPERATURES:

Store the DLAR-2B Component 1 and the 100X Renilla Substrate at -80 °C

Note: DLAR-2B Component 2 assay buffer and the Optional 5X Cell lysis buffer can be stored at RT if desired

All components should be thawed to room temperature before use.

#### Reagent Preparation:

- 1X CLR: Add 1 volume of 5X Cell lysis buffer to 4 volumes of distilled water. Mix well. Store at -20°C (≤1 6 months).
- DLAR-2B Component 1: Firefly luciferase assay reagent, Store at -20°C (≤1 month) or -80°C (≤1 year). Thaw and warm to RT just before use. This reagent has all the components for the Firefly luciferase assay. Thaw the reagent just before use, withdraw the required amount of reagent and freeze abck the rest.
- DLAR-2B Component 2: Renilla luciferase assay buffer(PLUS STOP), Store at -20 ° for 15 days or at - 80 ° C for 1 year.
- 100X RLAR substrate. To the required amount of DLAR-2B Renilla luciferase assay buffer, add 100X RLAR substrate (coelenterazine) to a final 1X concentration. For 10 ml of reagent add 100 ul of RLAR substrate to 9.9 ml of DLAR-2B Renilla luciferase (PLUS STOP) buffer. This buffer quenches the firefly luciferase activity and allows measurement of Renilla luciferase activity

**1. HTS (High throughput screening) Protocol (for use in 96-well plates (when performing the assay in 24-well, 12-well, 6-well plates fo 10 cm dishes please refer to Protocol 2 on the following page)**

The protocol below is for 96-well dishes, If performing the assay in 384 well dishes you can proportionately adjust the amoutns of reagetns added. For example when using 384 well dishes you can add 25 ul of DLAR-2B component 1 and 25 ul of DLAR-2b component 2

**Protocol:**

1. Assay for Firefly Luciferase by adding 100 ul of FLAR component directly to the cell culture supernatant of 96-well dishes. Read firefly luciferase activity. Note: The DLAR-2B component ! reagent has all the ingredients to measure firefly luciferase activity as well as ingredients to mediate immediate lysis of the cells so it can be added directly on top of cell culture media Wait for 5 minutes
2. Assay for Renilla Luciferase as per the RLAR-1 protocol (Add 100 ul of RLAR plus stop reagent formulated by mixing 50 ul of the coelenterazine stock solution (100x) with 5 ml of the Component 2 reagent buffer (RLAR plus stop buffer (this has the ingredients to stop the Firefly Luciferase. Note: We supply 5X lysis buffer and RLAR-1 buffer (without stop) separately in a bag labeled "OPTIONAL REAGENTS" as a courtesy in case you wish to assay for Renilla luciferase separately. If you follow the DLAR stop n glow protocol above the FLAR component has the lystic ingredients and cells should be lysed within 5 minutes of adding the reagent so you don't need a separate lysis step

**PROTOCOL 2 (Use when performing the assay in 12-well/24-well/6-well dishes/10 cm plates) Cell lysis protocol Intracellular Firefly luciferase/Renilla luciferase activity**

Lyse cells using our lysis buffer (Catalog no 5X CLR-01). Follow cell lysis protocol below. Assay as above using 5 ul to 10 ul of lysate  
Dilute the 5X CLR buffer (catalog #5XCLR-1)1:5 with water.

Cell Lysis

1. Remove growth media from cultured cells.
2. Rinse cultured cells in 1X PBS. Remove all rinse solution.
3. Dispense the recommended volume (below) of 1X CLR into each culture vessel.

Volumes of 1XCLR to be added to lyse cells

Plate Size	1XCCLR
10 cm dish	3 ml
6-well	600 ul
12-well	250 ul

24-well	100 ul
96-well	25 ul

Cell lysis:

Gently rock the culture dish at 400 rpm for 15 min to lyse cells

For HTS applications, the DLAR-2B Assay can be performed directly in the multi-well plate  
 (refer to HTS protocol on next page)

### Dual Luciferase Reporter Assay Protocol:

Assay performed in 96-well plates

Plate with 10-25 ul lysate

↓ Add 100 ul of DLAR-2B  
 Component1

Measure Firefly luciferase Activity

↓ Add 100 ul of RLAR (*plus STOP*) reagent

Measure Renilla luciferase Activity

### PRODUCT CITATIONS (selected) :

1. Albert NW. Subspecialization of R2R3-MYB Repressors for Anthocyanin and Proanthocyanidin Regulation in Forage Legumes. *Front Plant Sci.* 2015 Dec 23;6:1165. doi: 10.3389/fpls.2015.01165. PMID: 26779194; PMCID: PMC4689181.
2. Lafferty Dj et al (2022) The coordinated action of MYB activators and repressors controls proanthocyanidin and anthocyanin biosynthesis in vaccinium. *Front. Plant Sci.*, 24 June 2022 *Sec. Crop and Product Physiology*  
<https://doi.org/10.3389/fpls.2022.910155>
3. H. Zhou, K. Lin-Wang, H. Wang, C. Gu, A.P. Dare, R.V. Espley, H. He, A.C. Allan, Y. Han

---

**Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by NAC transcription factors**

Plant J., 82 (1) (2015), pp. 105-121, [10.1111/tpj.12792](https://doi.org/10.1111/tpj.12792)

**CUSTOM REAGENTS:** We can provide custom formulations to fit your HTS application. Call our tech support team at 1-619-562-1518 or email us [info@targetingsystems.com](mailto:info@targetingsystems.com) or [targetingsystems@gmail.com](mailto:targetingsystems@gmail.com). Please check out our website [www.targetingsystems.net](http://www.targetingsystems.net) for novel luciferase – based multiplexed assays which enable analysis of up to three promoter activities in the same group of transfected cells.