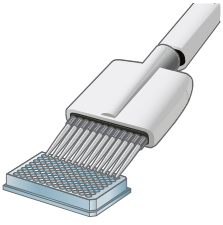
Nano-Glo® Dual-Luciferase® Reporter Assay System

INSTRUCTIONS FOR USE OF PRODUCTS N1610, N1620, N1630 AND N1650

Protocol for 96-Well Plates Using Multichannel Pipettes

1. Equilibrate plates to room temperature. Use a small enough volume of cells so that two equivalent volumes can be added to each well without risk of overflow. For 96-well plates, we recommend a starting volume of 80µl per well. Use an opaque, white tissue-culture plate to minimize cross-talk between wells and absorption of the emitted light.
2. **Measure firefly luciferase activity:**
   - Add a volume of ONE-Glo™ EX Reagent equal to the volume of culture medium.
   - Incubate the samples for at least 3 minutes. For best results, mix on an orbital shaker (300–600 rpm).
   - Measure firefly luminescence using settings specific to your instrument*.
3. **Measure NanoLuc® luciferase activity:**
   - Add a volume of NanoDLR™ Stop & Glo® Reagent equal to the original culture volume to each well and mix thoroughly. For best results, mix on an orbital shaker at 600–900 rpm for at least 3 minutes. Good results can also be achieved by pipetting up and down twice to mix.
   - After at least 10 minutes (including mixing time), measure NanoLuc® luminescence using settings specific to your instrument*.

*For 96-well plates on GloMax® instruments, integration times of 0.5–1 seconds are recommended.



Add ONE-Glo™ EX Luciferase Assay Reagent to the plate and mix.

Incubate at 20–25°C for 3 minutes–2 hours.

Measure firefly luminescence.

Add NanoDLR™ Stop & Glo® Reagent to the plate and mix.

Incubate at 20–25°C for 10 minutes–2 hours.

Measure NanoLuc® luminescence.

GloMax® Discover System

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Protocol for 96-Well Plates Using Dual-Sample Injection

The NanoDLR™ Assay can be performed using a luminometer equipped with a dual auto-injection system.

For every NanoDLR™ experiment run on a given instrument:

- Injector #1 should be dedicated to the delivery of ONE-Glo™ EX Reagent.
- Injector #2 should be dedicated to delivery of NanoDLR™ Stop & Glo® Reagent

Injector #2 should not be used to deliver firefly luciferase reagents or reagents with assay chemistries that use Ultra-Glo™ recombinant luciferase. See the *Nano-Glo® Dual-Luciferase® Reporter Assay Technical Manual* #TM426 for information on cleaning injectors after use.

Injecting at too high a speed may cause excessive foaming, which could increase signal variability or reduce signal intensity. Verify that injectors and injection settings provide good sample mixing and data reproducibility without excessive foaming. The default settings on GloMax® instruments generally work well.

Protocol for Dual-Injection Assays in 96-Well Format

1. Prime injector #1 with ONE-Glo™ EX Reagent and injector #2 with NanoDLR™ Stop & Glo® Reagent.
2. Remove plates from the incubator and equilibrate to room temperature. The samples should be in a starting volume of 80µl per well.
3. Inject all sample wells with 80µl of ONE-Glo™ EX Reagent from injector #1.
4. Incubate for 3 minutes.
5. Read the firefly luminescence in all sample wells using a 1-second integration time.
6. Inject all sample wells with 80µl of NanoDLR™ Stop & Glo® Reagent from injector #2.
7. Incubate for 5 minutes.
8. Read the NanoLuc® luminescence of all sample wells using a 1-second integration time.
9. Clean the dispensing lines as described in the *Nano-Glo® Dual-Luciferase® Reporter Assay Technical Manual* #TM426.

Protocol files for GloMax® instruments are available by request from Promega Technical Services: **techserv@promega.com**

Additional protocol information in Technical Manual #TM426 available online at: **www.promega.com/protocols/**

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