Introduction

To enable investigation of key cellular signaling pathways, Promega has developed a portfolio of bioluminescent reporter gene assays using Firefly and Renilla luciferases. In combination with best-in-class luciferase detection reagents, these genetic reporter systems enable interrogation of important cellular responses involved in cancer, inflammation, and CNS disease. To address specialized customer needs in our industrial and research markets, Promega has a new custom assay service team dedicated to applying these enabling technologies through strategic external research collaborations. The performance of this technology portfolio is presented, including novel applications of luciferase reporters to interrogation of cytokine, stress, and toxicity pathway responses.

A common pathway architecture among stress/cytokine signaling pathways

![Diagram of a common pathway architecture among stress/cytokine signaling pathways](image)

**Firefly Luciferase (luc2P) Reporter Performance in various cell backgrounds**

- **Stress / Growth**
- **Xenobiotic Stress**
- **Heat Shock**
- **Hypoxia**
- **ER Stress**
- **Oxidative Stress**
- **SMAD 3.4**
- **WH / p-lucanin**
- **Metal Stress**

Excellent performance in transients, pools, and stable lines

**Measurement of JAK/STAT Signaling using luc2P Reporter Assays**

![Image of Measurement of JAK/STAT Signaling using luc2P Reporter Assays](image)

**Protein stability reporters using Renilla Luciferase enable signaling pathway measurements in real-time**

![Image of Protein stability reporters using Renilla Luciferase enable signaling pathway measurements in real-time](image)

**NanO Luc: The Next Gen Luciferase**

- **Super bright**
- **Using HEK293 cells imaged by hand-held iPhone (50,000 cells/well; 96-well plate)**

**Superior sensitivity and temporal coupling for reporter applications**

**Materials and Methods.** Upper: (Left): HepG2 cells were transfected with varying concentrations of DNA encoding either RLuc or Fluc, followed by end-point measurements of luciferase activity via addition of Nano-Glo™ vs One-Glo™ reagents, respectively. Right: HEK cells were transfected with plasmid DNA constructs encoding RLuc or Fluc under the control of multiple repeats of an NFkB response element. Cells were stimulated with TNFα/Flp for five hours, and luminescence was measured using Nano-Glo™ or One-Glo™ reagents, respectively. Lower: (Left): HEK293 cells were transfected with plasmid DNA constructs encoding NFB/Rapid Response versions of NanO Luc (NlucP) or firefly luciferase (FlucP) followed by treatment with 100 ng/ml TNFα at time zero. Luminescence was measured at the indicated time points from replicate wells using Nano-Glo™ or One-Glo™, respectively. Right: HEK cells were transfected with plasmid DNA constructs encoding HSE-NlucP and HSE-FlucP followed by treatment with 500 nM of 17-allylamino-17-demethoxygeldanamycin (17-ANG) at time zero as above.

**Conclusions**

- Promega now offers fully validated firefly luciferase reporter vectors for key stress and cytokine signaling pathways
- Protein fusions of luciferases such as Renilla Luc can be used to monitor stress pathway responses in live cells and in real time.
- Due to its small size and increased sensitivity, NanoLuc technology offers advantages over other luciferase reporters.
- These reporter technologies can be engineered into the desired cellular context using Promega’s Custom Assay Service Team.

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