1. Abstract

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.

2. Toxicity Pathways: A Common Pathway Architecture

Latest Research Materials: Vectors and Cell Lines for Stress and Toxicity

Materials and Methods: Cytokine signaling factor (CNTF) is a 23 kDa neurokinin which is expressed in both the peripheral and central nervous system and provides hope for the possible clinical application in the treatment of human neurodegenerative diseases. Like other cytokines, CNTF exerts its biological effects through the activation of a multichain receptor complex consisting of a ligand-specific subunit (CNTFRα), gp130 and the LIF receptor β (LIFRβ). GloResponse™ SIE-Luc2P reporter is stably expressed in HepG2 cells where LIF and LIF-related cytokines stimulate transduction of intracellular signalling pathways following receptor dimerization. Cells were seeded in 96-well plate and incubated overnight at 37°C with 5% CO2. Next day, 10ul of 10X soluble CNTFRα was added to appropriate wells (or media, untreated wells) with subsequent 10ul of 10X CNTF dose addition. Cells were incubated 6 hours prior to One-Glo substrate (Promega, Cat.# A5610) addition. As shown in the data, in the present of exogenously added soluble CNTFRα receptor binding CNTFα receptor leads to CNTF at lower doses, consistent to the published data.

6. CASE STUDY #2: Leveraging SRE-Luc2P/HEK293 Reporter Cell Line to Bioassay Development

Materials and Methods: Global Response™ SRE-Luc2P/HEK293 cells were stably transfected with pF5A DNA encoding humanized SRE reporter DNA and is resistant to 800 μM G418. Cells were transiently transfected with 200 nM dual luciferase substrate and 20 μg DNA with 5 μg pRL-TK internal control DNA using Lipofectamine™ 2000 transfection reagent (Invitrogen). Cells were treated with different levels of isoforms of luciferase activity. 24 hours post-transfection cell media was replaced with CO2 independent medium supplemented with 20% FBS (Gibco™ ambion). Cells were then stimulated with the isokaryoid isolated against indicated, for the time indicated. Luminescence was then measured in real-time using a Glomax Multi-Plate reader set to 5 minute measurement intervals. For data normalization, RLU’s of each sample were normalized to the RLU values immediately after stimulation for that sample. Figure 3: Comparison of pharmacology between luciferase fusion and reporter gene assay for measuring hypoxia response (top) and TNF 

9. Conclusions

- Promega now offers fully validated Firefly luciferase reporter vectors for key toxicity and cytokine signaling pathways
- Modular solutions exist for the investigation of endogenous and exogenous receptor biology using GloResponse™ stable cell lines
- Novel pathway analysis tools are available using luciferase fusion technologies
- Promega’s Custom Assay Service (CAS) team provides expertise in generation of custom vectors or stable cell lines encoding bioluminescent reporters