Monitor changes in gene expression using luminescence
The HTS-compatible TurboLuc Luciferase One-Step Glow Assay.

The study of complex cellular signaling pathways requires powerful, specific tools to accurately monitor changes in gene activation or repression. Because of their ultrasensitive detection capabilities and wide dynamic range, luciferase-based reporter gene assays are widely used to measure the activity of promoters and other transcriptional regulatory elements, as well as the effects of activators and inhibitors. However, the "flash" type enzyme kinetics associated with the conventional ultrasensitive luciferase assay format requires specialized equipment for measurement; and the enzymatic nature of the reporter makes these assays susceptible to modulation by a variety of small-molecule compounds found in screening collections, preventing their adoption in high-throughput screening (HTS) applications.

Meet the innovative TurboLuc luciferase
The Thermo Scientific™ TurboLuc™ Luciferase One-Step Glow Assay was designed to measure luciferase activity in mammalian cells with the addition of a single reagent, making it ideal for HTS applications. This one-step homogeneous assay employs a novel 16 kDa ATP-independent luciferase (TurboLuc16, or Tluc16)—the smallest luciferase described to date—derived from a marine copepod of the genus Metridia. The wild-type luciferase was modified to reduce its size, increase its brightness, and enable its efficient intracellular expression (Figure 1A). The TurboLuc gene was further modified to include patented dual-destabilization elements that reduce nonspecific accumulation of the TurboLuc mRNA and protein in cells (Figure 1B), enhancing the responsiveness and sensitivity of the assay (Figure 1C). With the addition of coelenterazine, Tluc16 produces intense blue-luminescent light that is stable over the detection period, enabling measurement of very minute amounts of luciferase activity using a standard luminometer, HTS instrument, or other automated detection platform.

Robust performance in high-throughput formats
When compared with the Promega NanoLuc™ system, the TurboLuc Luciferase One-Step Glow Assay shows brighter signals (Figure 2A), as well as a similarly stable signal over time (Figure 2B). With its increased luminescence response, stable glow kinetics, and simple one-step protocol, the TurboLuc luciferase assay has been specifically designed for use in HTS applications and other laboratory automation formats and should prove especially beneficial when detecting low or transient levels of gene expression. Figure 3 demonstrates the use of the...

Figure 1. Optimizing the TurboLuc16 luciferase for high-throughput screening applications. (A) Mutagenesis of the wild-type luciferase from Metridia has produced a luciferase reporter enzyme with desirable glow kinetics. HEK 293 cells were transfected with plasmids containing the dual-destabilized TurboLuc16 luciferase (Tluc16-DD) gene or the non-optimized luciferase gene, and luciferase activity was measured using the TurboLuc Luciferase One-Step Glow Assay Kit over a 1 hr period. (B) The presence of dual-destabilization (DD) elements reduces accumulation of luciferase in cells. Luciferase activity was measured in HEK 293 cells transfected with plasmids containing either the dual-destabilized Tluc16 gene (pMCS minP-Tluc16-DD, Cat. No. 88232) or the nondestabilized Tluc16 gene (pMCS minP-Tluc16, Cat. No. 88236) under the control of an optimized minimal core promoter (minP) designed for the measurement of nonspecific expression of the luciferase reporter. (C) The TurboLuc16 luciferase with dual-destabilization technology shows improved responses in biological assays. Luciferase activity was measured in HEK 293 cells transfected with plasmids containing either the dual-destabilized Tluc16 gene (pCRE-Tluc16-DD, Cat. No. 88247) or the nondestabilized Tluc16 gene (pCRE-Tluc16) under the control of a combination of an optimized minimal core promoter and five tandem repeats of the cAMP response element (CRE). Results are displayed as fold induction of Tluc16 activity in cells treated with 10 µM forskolin (to raise cAMP levels) relative to untreated cells.
TurboLuc assay to generate dose-response curves using an HTS platform. After transfection with a plasmid containing the Tluc16-DD gene under the control of NF-κB response elements, HEK 293 cells were plated into multiple 384-well plates and then stimulated with a series of TNF-α concentrations to generate dose-response curves at different cell numbers. The luminescence response was reproducibly measured over several orders of magnitude in TNF-α concentration and one order of magnitude in cell concentration. The combination of brightness, responsiveness, and stable glow characteristics makes the TurboLuc system an ideal platform for reporter gene assays for HTS applications.

**TurboLuc expression vectors:**
**Choose from a variety of transcriptional regulation elements**

The Tluc16 luciferase expression vectors are offered in a variety of configurations to enable different experimental workflows. All of these cloning vectors contain unique multiple cloning sites (MCS) to accept transcriptional regulators such as promoters or response elements. The miniP-Tluc16 vectors contain a core minimal promoter (miniP) designed for studying the regulation of transcription by elements lacking promoter activity. The Tluc16-DD expression vectors incorporate the patented dual-destabilization (DD) technology. To minimize assay variability, stable cell lines selected for genetically homogeneous expression are desirable. These stable cell lines can be generated either through positive selection using Tluc16 vectors containing the gene for hygromycin resistance or through the use of cell engineering tools such as the BacMam gene delivery and expression system, Invitrogen™ Jump-In™ technology, or other gene-editing methods.

**Learn about the TurboLuc Luciferase One-Step Glow Assay**

The TurboLuc™ Luciferase One-Step Glow Assay Kit contains an assay buffer and substrate solution that have been specifically developed to function in a one-step homogeneous assay format that is amenable to laboratory automation methods. Find out more about the TurboLuc Luciferase One-Step Glow Assay, as well as our selection of over 10 different Tluc16 expression vectors, at thermofisher.com/turbolucbp73.