ViaScript™ LgBiT mRNA Delivery System

Instructions for Use of Products NE1120, NE1130 and NE1140.

Quick Protocol

Proteins fused to the 11-amino-acid HiBiT tag (e.g., via CRISPR-mediated gene editing) can be kinetically monitored in live cells expressing LgBiT protein, which binds with high affinity to HiBiT to reconstitute NanoBiT® luciferase. The ViaScript™ LgBiT mRNA Delivery System[(a)] enables uniform, high-efficiency transfection of LgBiT mRNA, facilitating live-cell kinetic assays to monitor changes in HiBiT-tagged protein levels.

For more detailed information about mRNA transfection, consult the *ViaScript™ mRNA Transfection Reagent Technical Manual* #TM761.

Product	Size	Cat.#
ViaScript™ LgBiT mRNA Delivery System plus eGFP Includes: • 0.1ml ViaScript™ Reagent • 11ml ViaScript™ Buffer • 0.1ml LgBiT mRNA, 0.2mg/ml • 0.1ml eGFP mRNA, 0.2mg/ml	10 plates	NE1120

Product	Size	Cat.#
ViaScript™ LgBiT mRNA Delivery System Includes: • 0.1ml ViaScript™ Reagent • 11ml ViaScript™ Buffer • 0.1ml LgBiT mRNA, 0.2mg/ml	10 plates	NE1130

Product	Size	Cat.#
ViaScript™ LgBiT mRNA Delivery System Includes: • 5 × 0.1ml ViaScript™ Reagent • 5 × 11ml ViaScript™ Buffer • 5 × 0.1ml LgBiT mRNA, 0.2mg/ml	5 × 10 plates	NE1140

Storage Conditions: Store ViaScript™ Reagent at +2°C to +10°C. Do not freeze or store below 0°C. Close the cap tightly after use to prevent evaporation. ViaScript™ Buffer can be stored at +2°C to +10°C or room temperature. Bring ViaScript™ Buffer to room temperature before use. Store eGFP mRNA and LgBiT mRNA below –65°C and dispense into aliquots after thawing to minimize freeze-thaw cycles.

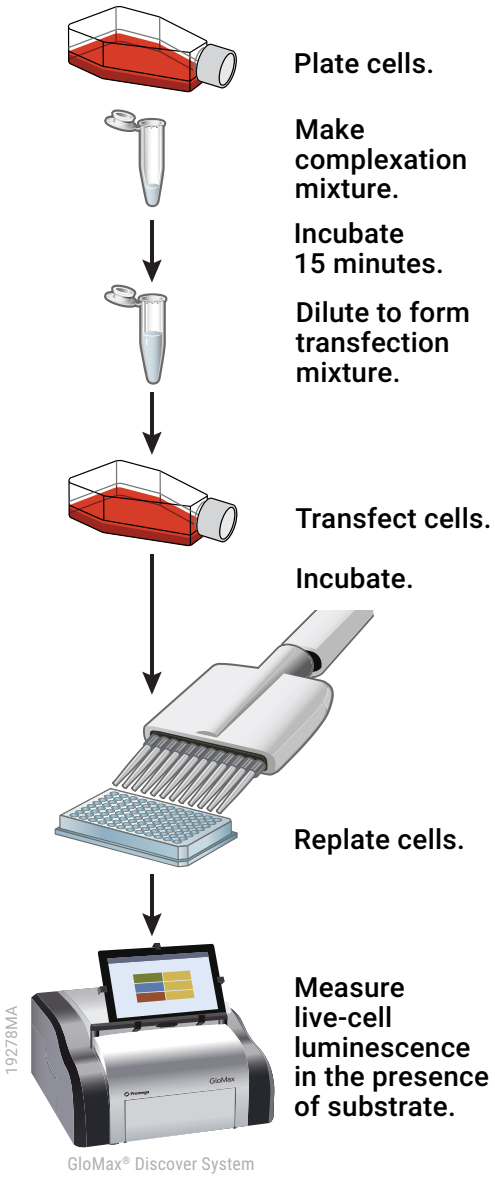

Figure 1. Overview of the ViaScript™ LgBiT mRNA Delivery workflow.

Additional protocol information is in Technical Manual #TM761, available online at: **www.promega.com**

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Instructions for Use of Products NE1120, NE1130 and NE1140.

Quick Protocol

The following protocol describes the transfection of cells in a T75 flask, followed by replating into 96-well assay plates for kinetic monitoring of HiBiT levels. For smaller or larger culture vessels, adjust the protocol volumes proportionally based on the medium volume.

Bulk LgBiT mRNA Transfection of Cells Using ViaScript™ Reagent

1. Plate the cells in a T75 flask.
   - **Adherent cells:** Plate the cells the day before transfection so they will be 50–80% confluent the next day ($0.5–1.5 \times 10^6$ cells in 10ml of medium).
   - **Suspension cells:** Plate the cells the same day as transfection at $0.2–1 \times 10^7$ cells in 10ml of medium.
2. Prepare the complexation mixture as follows:
   - Add 80µl of ViaScript™ Buffer to a sterile RNase-free tube.
   - Add 10µl of 0.2mg/ml LgBiT mRNA and mix thoroughly by pipetting.
   - Add 10µl of 1mg/ml ViaScript™ Reagent and mix thoroughly by pipetting.

   Note: Transfect adherent cells in the morning to replate later in the day; transfect suspension cells in the afternoon for highest expression the following day.
3. Incubate the mixture for 15 minutes at room temperature. Do not incubate longer than 30 minutes.
4. Form the transfection mixture by combining 100µl of the complexation mixture with 900µl of ViaScript™ Buffer, and mix gently.
5. Transfect the cells by adding the 1ml of transfection mixture to the T75 flask containing 10ml of medium and mixing gently.
6. Incubate the cells at 37°C for 4–8 hours (adherent cells) or 16–24 hours (suspension cells), depending on the timing of replating.

Replating Cells for Kinetic Live-Cell Measurement of HiBiT Expression

1. Replate cells and replace medium with buffered medium containing a Nano-Glo® Live Cell Substrate (Cat.# N2011, N2570, N2580), e.g., CO_2-independent medium + 10% FBS containing Nano-Glo® Endurazine™ Live Cell Substrate for ≥24 hour kinetic measurements.
   - **Adherent cells**

     After transfecting for 4–8 hours, dissociate the cells in the T75 flask, centrifuge and resuspend to the desired density. Replate the cells into 96-well assay plates and incubate overnight at 37°C.

     On the day of the experiment, replace the medium with buffered medium containing the chosen Nano-Glo® Live Cell Substrate.
   - **Suspension cells**

     After transfecting for 16–24 hours, remove the cells from the T75 flask, centrifuge and resuspend to the desired density in buffered medium containing the chosen Nano-Glo® Live Cell Substrate. Consider resuspending cells in medium without substrate to a higher cell density, counting the cells and then diluting them into additional medium containing the appropriate amount of substrate. Replate the cells into 96-well assay plates.
2. Measure luminescence kinetically in a luminometer set to 37°C, with the plate lid on to prevent evaporation.
3. Once the signal has stabilized (typically 2–3 hours for Endurazine™ substrate), add test compounds (e.g., PROTACs or other degraders).
4. Measure luminescence kinetically over 24 hours or for the desired duration.

Example of Kinetic Live-Cell HiBiT Measurement

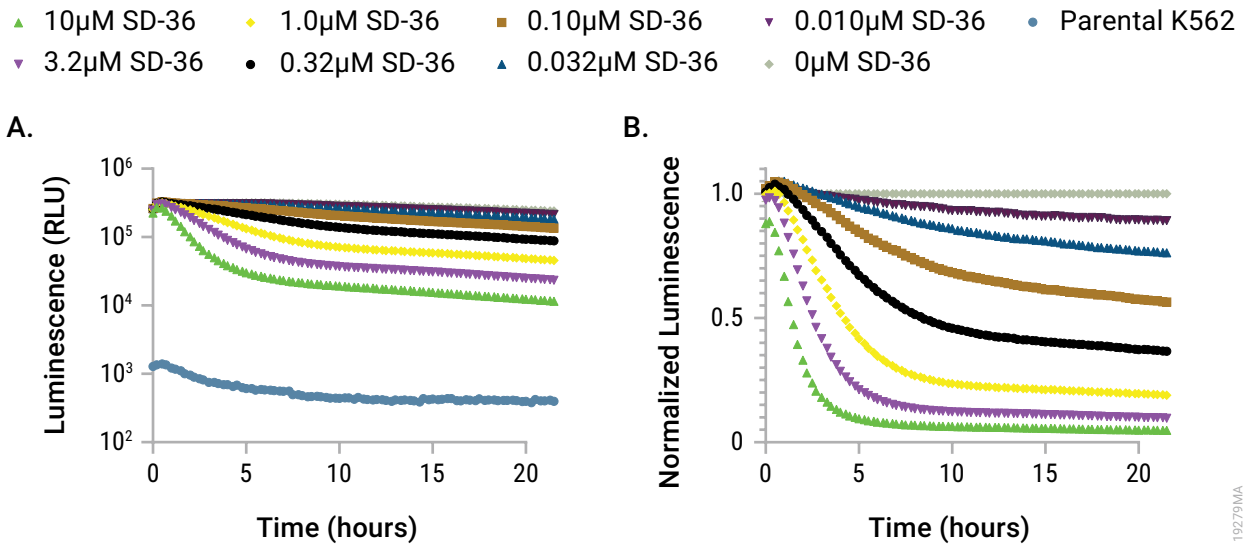

Figure 2. Induced degradation of STAT3-HiBiT in K562 cells transfected with LgBiT mRNA. A CRISPR-edited K562 cell line expressing endogenously tagged STAT3-HiBiT was bulk transfected with LgBiT mRNA using ViaScript™ Reagent, as described in the protocol. The parental K562 cell line, which does not express HiBiT, was transfected under the same conditions for comparison. After resuspending and equilibrating cells in medium containing Endurazine™ substrate, the STAT3-HiBiT line was treated with a titration of the STAT3 PROTAC degrader SD-36. **Panel A** displays the raw luminescence values, demonstrating the high signal-to-background ratio in the STAT3-HiBiT line relative to parental cells. **Panel B** presents the luminescence data normalized to the vehicle control, showing the effect of the degrader on the relative STAT3 protein levels over time.

Optimizing Signal-to-Background Levels

In some cell lines, high LgBiT expression can result in background luminescence in the absence of HiBiT. Diluting LgBiT mRNA into a carrier RNA, such as eGFP mRNA (provided with Cat.# NE1120), can reduce LgBiT expression and improve the signal-to-background ratio. For detailed instructions, refer to Section 5.E of the *ViaScript™ mRNA Transfection Reagent Technical Manual* #TM761.