Autophagy LC3 HiBiT Reporter Assay System

Instructions for Use of Products GA1040, GA1050 and GA2550.

Promega

Quick Protocol

The following protocol is for general detection of autophagy inducers and inhibitors. For a detailed protocol including reporter cell propagation, optimized autophagy inhibitor detection and guidance on data interpretation, see the *Autophagy LC3 HiBiT Reporter Assay System Technical Manual #TM535*. For a detailed protocol on the Nano-Glo® HiBiT Lytic Reagent, see the *Nano-Glo® HiBiT Lytic Detection System Technical Manual #TM516*.

Treatment of Autophagy LC3 HiBiT Reporter Cells

1. Plate cell suspension (80µl) containing 20,000 HEK293 Autophagy LC3 HiBiT Reporter Cells or 8,000 U2OS Autophagy LC3 HiBiT Reporter Cells into each inner-60 well of sterile, white 96-well plates. Dispense outer wells with 80µl/well cell culture medium without cells. Allow cells to attach overnight before treatment.
2. Prepare the following working solutions in prewarmed (22–37°C) cell culture medium.

	Vehicle	1,000X Test Compound	1,000X Reference Inducer	1,000X Reference Inhibitor	Cell Culture Medium	Total Volume
Vehicle Dilution	2µl				398µl	400µl
5X Test Compound		2µl			398µl	400µl
5X Reference Inducer			2µl		398µl	400µl
5X Reference Inhibitor				2µl	398µl	400µl

3. To replicate assay wells containing Autophagy LC3 HiBiT Reporter Cells in 80µl cell culture medium, add 20µl treatment volumes as indicated in the following table.

	Cell Volume	Vehicle Dilution	5X Test Compound	5X Reference Inducer	5X Reference Inhibitor	Total Well Volume
Vehicle	80µl cells	20µl				100µl
Test Compound	80µl cells		20µl			100µl
Reference Inducer	80µl cells			20µl		100µl
Reference Inhibitor	80µl cells				20µl	100µl
No-Cell Background Control	80µl culture medium only	20µl				100µl

4. Return assay plate(s) to a 37°C, 5% CO_2 incubator for the desired treatment time (typically 5–24 hours).

Detection Using Nano-Glo® HiBiT Lytic Reagent

1. Prepare the required volume of Nano-Glo® HiBiT Lytic Reagent for the particular study needs by diluting the LgBiT Protein 1:100 and the Nano-Glo® HiBiT Lytic Substrate 1:50 into an appropriate volume of room-temperature Nano-Glo® HiBiT Lytic Buffer in a new tube. Mix by inversion.
2. Remove treated assay plate(s) from the incubator and equilibrate to room temperature for 10–15 minutes.
3. Add 100µl of Nano-Glo® HiBiT Lytic Reagent to each assay well and mix the samples by placing the plate on an orbital shaker (300–400rpm) for 2 minutes.
4. Wait at least 10 minutes total, including shaking time, for equilibration of LgBiT and HiBiT binding in the lysate. Measure luminescence using settings specific to your instrument. When using 96-well plates on the GloMax® instruments, we recommend integration times of 0.5–2 seconds. Assay signal half-life >3 hours.

Additional protocol information is in Technical Manual #TM535, available online at: **www.promega.com**

