Using the NanoBiT® PPI System with GloMax® Discover to Detect Protein-Protein Interactions

Promega Corporation

Materials Required

- FRB-LgBiT Control Vector (Cat# N2016)
- FKBP-SmBiT Control Vector (Cat# N2016)
- Nano-Glo® Live Cell Assay (Cat# N2011)
- GloMax® Discover System (Cat.# GM3000)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- White, 96-well Tissue Culture assay plates (Corning Cat.# 3917)
- Rapamycin Cat.# R8781/lot 014M4097V

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocols: *GloMax Discover System Technical Manual* #TM397 and *NanoBiT™ Protein:Protein Interaction System Technical Manual* #TM461, available at: available at: **www.promega.com/protocols/**

NanoLuc® Binary Technology, or NanoBiT®, is a complementation system based on two NanoBiT® Luciferase subunits. The large subunit, (LgBiT; 17.6 kDa), has enhanced structural stability. The small subunit (SmBiT; 11 amino acids), was developed for use in protein:protein interaction (PPI) assays. In NanoBiT® PPI assays, LgBiT and SmBiT are fused to two interacting target proteins. When these proteins interact, LgBiT and SmBiT are brought into close proximity, allowing structural complementation to give a bright, luminescent enzyme. The weak affinity of LgBiT and smBiT for self-association (KD = 190 µM) minimizes assay background and reduces experimental artifacts. Interactions between proteins can be monitored in real time in living cells using the NanoBiT® PPI Systems.

This Application Note presents an example protocol using the NanoBiT® PPI System and GloMax® Discover to detect rapamycin-inducible FKBP:FRB protein interactions. Dose response of rapamycin was evaluated, and kinetics of the protein association were followed in real time.

NanoBiT® Assay Protocol

Day 1 (Plate Cells)

1. Use the inner 60 wells of a 96-well plate. Plate HEK293 cells in complete medium (DMEM + 10% FBS) in 100µl/well at a concentration of 1×10^5 cells/ml.
2. Incubate cells overnight at 37°C, 5% CO_2.
3. Add 200µl DPBS to the outside wells.

Day 2 (Transfection)

1. Dilute plasmid DNA encoding FRB-LgBiT Control Vector and FKBP-SmBiT Control Vector in OptiMEM® I Reduced Serum Medium (Life Technologies Cat.# 11058) to 6.25ng/µl for each construct.
2. Add FuGENE® HD Transfection Reagent at a 3:1 lipid-to-DNA ratio.
3. Incubate at ambient temperature for 10 minutes.
4. Add 8µl of the transfection mix to each well.
5. Incubate at 37°C, 5% CO_2 for ~24 hrs.

Day 3 (Assay):

1. Exchange medium to 100µl/well pre-warmed Opti-MEM® I Reduced Serum Medium.
2. Incubate in 37°C incubator (5% CO_2) for 30 minutes.
3. Equilibrate Nano-Glo® LCS Dilution Buffer to room temperature.
4. Make Nano-Glo® Live Cell Reagent by combining 1 volume of Nano-Glo® Live Cell Substrate with 19 volumes of Nano-Glo® LCS Dilution Buffer (a 20-fold dilution), creating a 5X stock to mix with cell culture medium.
5. Prepare 13.5X stocks of rapamycin for dose response by diluting compound in Optimem:
   Final concentration in nM: 100.00, 31.8, 10, 3.18, 1.0, 0.318, 0.10, 0.031, 0.01, vehicle control (DMSO).
6. Prepare GloMax® Discover by selecting the protocol for Nano-Glo® Live Cell Assay. Select the inner 60 wells to be measured.
7. Add 25µl of 5X Nano-Glo® Live Cell Reagent per well and place plate in GloMax® Discover.
8. Protocol is set as follows:
   1. Measure every minute at 0.5 sec/well for 30 minutes (30 reads).
   2. A prompt to add compound will appear. Add 10µl of a 13.5X stock of rapamycin titration.
   3. A prompt to close door and start reads will appear. The plate will be read 30 times at 1-minute intervals.

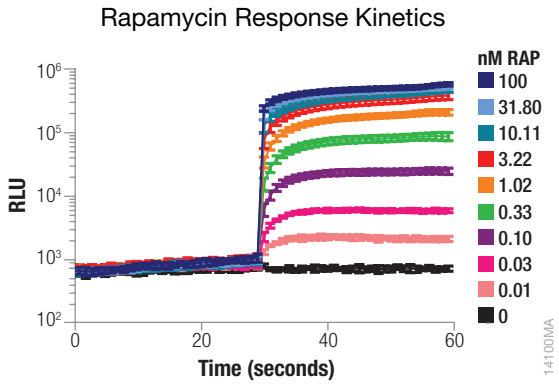

Figure 1. Rapamycin response kinetics. Using the GloMax® Discover and the Nano-Glo® Live Cell Assay program, signal was measured from cells expressing the FRB:FKBS NanoBiT® control pair for 30 minutes before a 13.5X titration of rapamycin was added and luminescence measured for an additional 30 minutes.

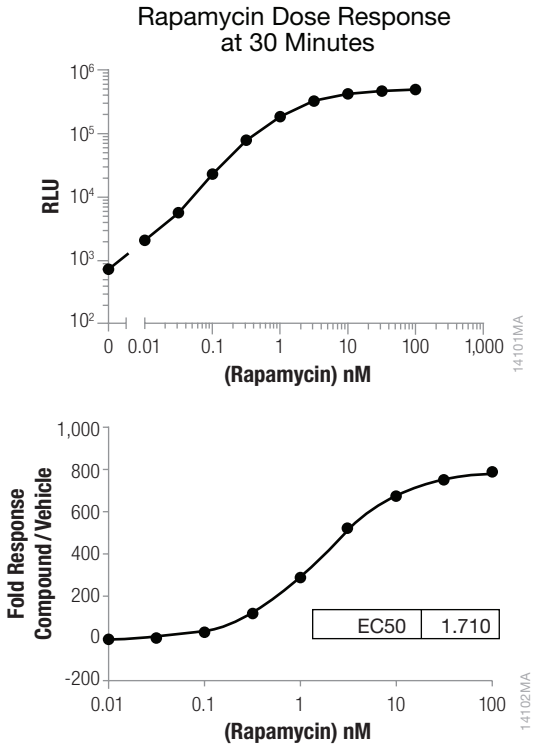

Figure 2. Average signal and fold response after 30 minutes rapamycin exposure.

Conclusion

The GloMax® Discover was used to detect protein-protein interactions using the NanoBiT® PPI Assay with the FKBP:FRB model system. PPI dynamics were followed in real-time and the expected pharmacology demonstrated.

GoMax® Discover System

The GloMax® Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax® Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.

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