Monitoring intracellular protein interactions using NanoLuc® Binary Technology (NanoBiT™)

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1. Introduction

Protein-protein interactions (PPIs) are essential to the cellular signal transduction pathways that contribute to cancer. Although numerous approaches exist to monitor PPIs in vitro, methods for intracellular detection have been more limited. We developed NanoLuc® Binary Technology (NanoBIT), a two-subunit system based on NanoLuc® luciferase that can be applied to the intracellular detection of PPIs. Large BIT (LgBiT, 18 kDa) and Small BIT (SmBiT, 11 amino acid peptide) subunits are expressed as fusions to proteins of interest, where PPI facilitates subunit complementation to give a bright, luminescent enzyme. Unlike related approaches where an enzyme or protein is simply split, LgBiT was independently optimized for structural stability and SmBiT was selected from a peptide library specifically for the PPI application. The result is a subunit pair that weakly associates (Kd = 190 μM) yet still maintains 30% of the activity of full-length NanoLuc at saturation. In contrast to many split systems, the LgBiT:SmBiT interaction is reversible, allowing the detection of rapidly dissociating proteins. PPI dynamics can be followed in real-time in living cells using the Nano-Glo® Live Cell Reagent, a non-lytic detection reagent containing the cell-permeable firefly luciferase substrate. Advantages over split systems include better sensitivity, reversibility, fusion to a peptide or a small, structurally stable protein domain, real-time measurements using a non-lytic assay format, and subunits with reduced affinity for self-association.

2. NanoBiT overview

LgBiT and SmBiT are fused to proteins A & B
• A:B interaction facilitates LgBiT:SmBiT interaction, generating a bright luminescent enzyme
• LgBiT:SmBiT with low affinity (Kd = 190 μM), limiting non-specific association and reducing assay background
• LgBiT:SmBiT interaction is reversible (kon = 500 M⁻¹ sec⁻¹; koff = 0.2 sec⁻¹)
• LgBiT evolved for increased structural stability making it a better fusion partner
• Non-lytic assay format allows real-time measurements of protein interaction dynamics for 1-2 hrs

3. Real time kinetics of PPI interaction dynamics

• Transient expression of LgBiT-PRKACA & LgBiT-PRKAR2A in HEK293 cells
• Modulators of intracellular cAMP added sequentially at indicated time points
• Inverse correlation for NanoBiT vs. GloSensor cAMP 22F (cAMP biosensor)
• NanoBiT can monitor reversible PPIs in real time

4. GPCR interaction with β-arrestin-2

• Stable expression of BRAF-LgBiT & CRAF-SmBiT via bi-directional CMV promoter
• Recombine mediated, single copy integration into HEK293 genome
• Expected rank order potency observed for BRAF inhibitors that promote dimerization
• β values <0.5 in 384-well format using manual dispensing
• NanoBiT can be miniaturized to 384- & 1536-well formats

5. RAF dimerization using stable expression

• Transient expression of ADRB2-LgBiT/SmBiT-ARRB2 or AVPR2-SmBiT/LgBiT-ARRB2 in HEK293 cells
• Saturation ISO or AVP added at time zero
• Expected transient interaction seen for ADRB2-ARRB2 (class A receptor)
• Expected stable interaction seen for AVPR2-ARRB2 (class B receptor)
• NanoBiT does not interfere with endogenous biology

6. Nuclear hormone receptor dimerization

• Transient expression of LgBiT-AR & SmBiT in HEK293 cells
• R181T agonist added at time zero, which induces AR dimerization
• CPR data plotted at 30 minutes
• Validated for GR homodimerization as well
• NanoBiT can monitor nuclear hormone receptor dimerization in real time

7. Agonist-induced RTK dimerization

• The cytoplasmic domain of HER1, 2 & 3 was replaced with tTR or-βT
• Transient expression in U2OS cells
• Saturation EGF or NRG1 added at time zero
• The expected selectivity profile was seen for EGF and NRG1
• NanoBiT can monitor RTK homo- or heterodimerization in real time

8. Quantifying agonist binding via dimerization

• HER truncations used as described above
• Transient expression in U2OS cells
• Varying EGF or NRG1 added at time zero
• CPR data at 1 minute
• Quantify agonist interaction with RTK via dimerization

9. Summary

NanoBiT is extremely bright
• Fusion partners can be expressed at very low levels, minimizing potential artifacts
• 100-1,000 fold brighter than split firefly luciferase

NanoBiT components are small & stable
• LgBiT, 18 kDa; SmBiT, 11 amino acids
• LgBiT evolved for increased structural stability, providing a stable fusion partner

NanoBiT is reversible
• Monitor both protein association and dissociation in real time

NanoBiT offers experimental flexibility
• Monitor protein interaction dynamics at a single time point or continuously for 1-2 hours
• Room temperature or 37 °C measurements
• Validated in 96-, 384-, & 1536-well formats

Check http://www.promega.com/nanobit for new NanoBiT PPI expression vectors

For more information on NanoBiT, please see ACS Chemical Biology, 11(2), p. 490-408