# NanoBRET™ Interaction Assays for Protein Degradation

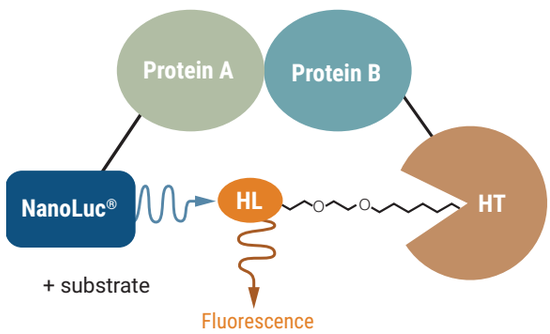
Proteins targeted for degradation via the ubiquitin proteasome system (UPS) require efficient ubiquitin conjugation prior to proteasome trafficking. For target protein degradation initiated by degrader compounds such as PROTACs or molecular glues, formation of a ternary complex consisting of the target protein/degrader compound/E3 ubiquitin ligase component is the first key mechanistic step. Therefore, this ternary complex formation represents a critical step in development of effective degrader compounds.

NanoBRET™ technology can be used to monitor key interactions along the UPS within live cells. The protein being monitored for degradation is fused to NanoLuc® luciferase or the HiBiT luminescent tag and serves as the bioluminescent energy donor. The interacting partner along the UPS is fused to the HaloTag® protein which serves as the fluorescent acceptor. Because the bioluminescent donor signal is measured independently, the abundance of the target protein can be monitored separately in the same assay, and the interaction can be measured over time even with target protein loss.

## *Advantages:*

- Obtain physiologically relevant results with either endpoint or real-time, kinetic analysis by monitoring ternary complex formation, ubiquitination, or recruitment to the proteasome in live cells.
- Measure target protein interactions with ectopically expressed or endogenously tagged proteins.
- Gain mechanistic understanding of key interactions critical for target protein degradation.
- Understand impact of compound structure on ternary complex formation and productivity.

## *NanoBRET™ Assay Principle*




**HL:** HaloTag® NanoBRET™ 618 Ligand

**HT:** HaloTag® protein

## *Monitor Ternary Complex Formation and Target Protein Abundance in Same Assay*

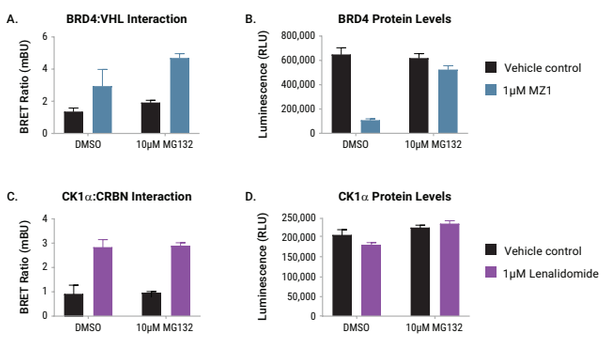



**Figure 1. Measuring PROTAC and molecular glue-induced ternary complexes and impact on BRD4 protein levels.** Ternary complex formation for both a PROTAC (NanoLuc®-BRD4:HaloTag®-VHL fusion proteins) and a molecular glue (NanoLuc®-CK1α:HaloTag®-CRBN fusion proteins) was measured using transiently transfected vectors in HEK293 cells. Since the target being degraded in both assays is the bioluminescent donor protein, its protein level is monitored separately in the same assay. Treatment with the proteasome inhibitor MG132 can be included to increase assay window but omitted when determining the effect on target protein levels.

## *Assay Protein Ubiquitination and Proteasomal Recruitment Kinetics Following Compound Treatments*

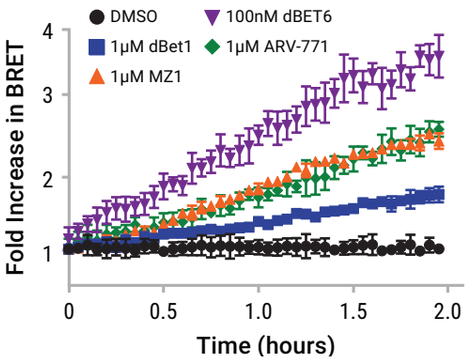



**Figure 2. Endogenous BRD4 ubiquitination kinetics following treatment with various PROTAC compounds.** BRD4 was HiBiT-tagged at the endogenous locus using CRISPR/Cas9 gene editing in a HEK293 LgBiT stable cell line to create the bioluminescent donor. HaloTag®-Ubiquitin was transiently expressed and NanoBRET™ signal measured using NanoBRET™ Nano-Glo® Kinetic Detection System following treatment with various PROTAC compounds as indicated. Varying patterns of ubiquitination are observed depending on the PROTAC being used to target BRD4 for degradation.

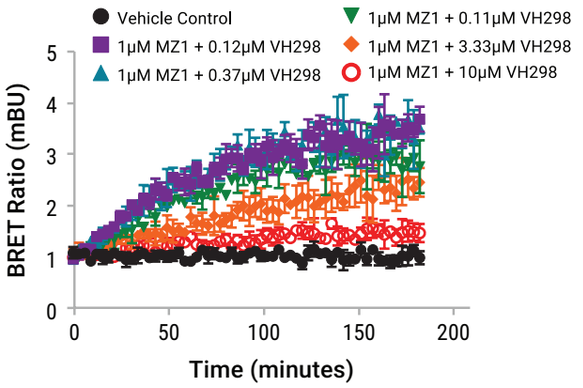



**Figure 3. BRD4 proteasomal recruitment kinetics following compound treatment.** NanoLuc®-BRD4 and HaloTag®-PSMD3 fusion vectors were transiently transfected into HEK293 cells. NanoBRET™ signal was measured using NanoBRET™ Nano-Glo® Kinetic Detection System and showed the expected kinetic increase in proteasome trafficking when MZ1, a VHL-mediated PROTAC, was added. Assay specificity was demonstrated by adding increasing levels of VH298, which binds VHL and blocks the PROTAC-mediated ternary complex and BRD4 ubiquitination.

Learn more about assays for Targeted Protein Degradation at: **www.promega.com/NanoBRETDegradation**

## Ordering Information

| Product | Size | Cat.# |
|---|---|---|
| NanoBRET™ Ubiquitination Starter Kit | 1 each | N2690 |
| NanoBRET™ VHL Ternary Complex Starter Kit | 1 each | N2700 |
| NanoBRET™ CRBN Ternary Complex Starter Kit | 1 each | N2720 |
| NanoBRET™ Proteasomal Recruitment Starter Kit | 1 each | N2730 |

Starter systems contain NanoLuc® cloning vectors to create target protein fusions, HaloTag® fusion vectors specific for interaction assay, NanoLuc®-BRD4 positive control vector, HaloTag® negative control vector, NanoBRET™ Nano-Glo® Detection System, 200 assays. Individual components are also available separately.

| Product | Size | Cat.# |
|---|---|---|
| NanoBRET™ Nano-Glo® Detection System | 200 assays | N1661 |
| | 1,000 assays | N1662 |
| | 10,000 assays | N1663 |
| NanoBRET™ Nano-Glo® Kinetic Detection System | 200 assays | N2583 |
| | 1,000 assays | N2584 |
| | 10,000 assays | N2585 |

NanoBRET™ Nano-Glo® Detection System allows live cell endpoint measurement. NanoBRET™ Nano-Glo® Kinetic Detection System allows real-time, live cell measurements lasting several hours.

