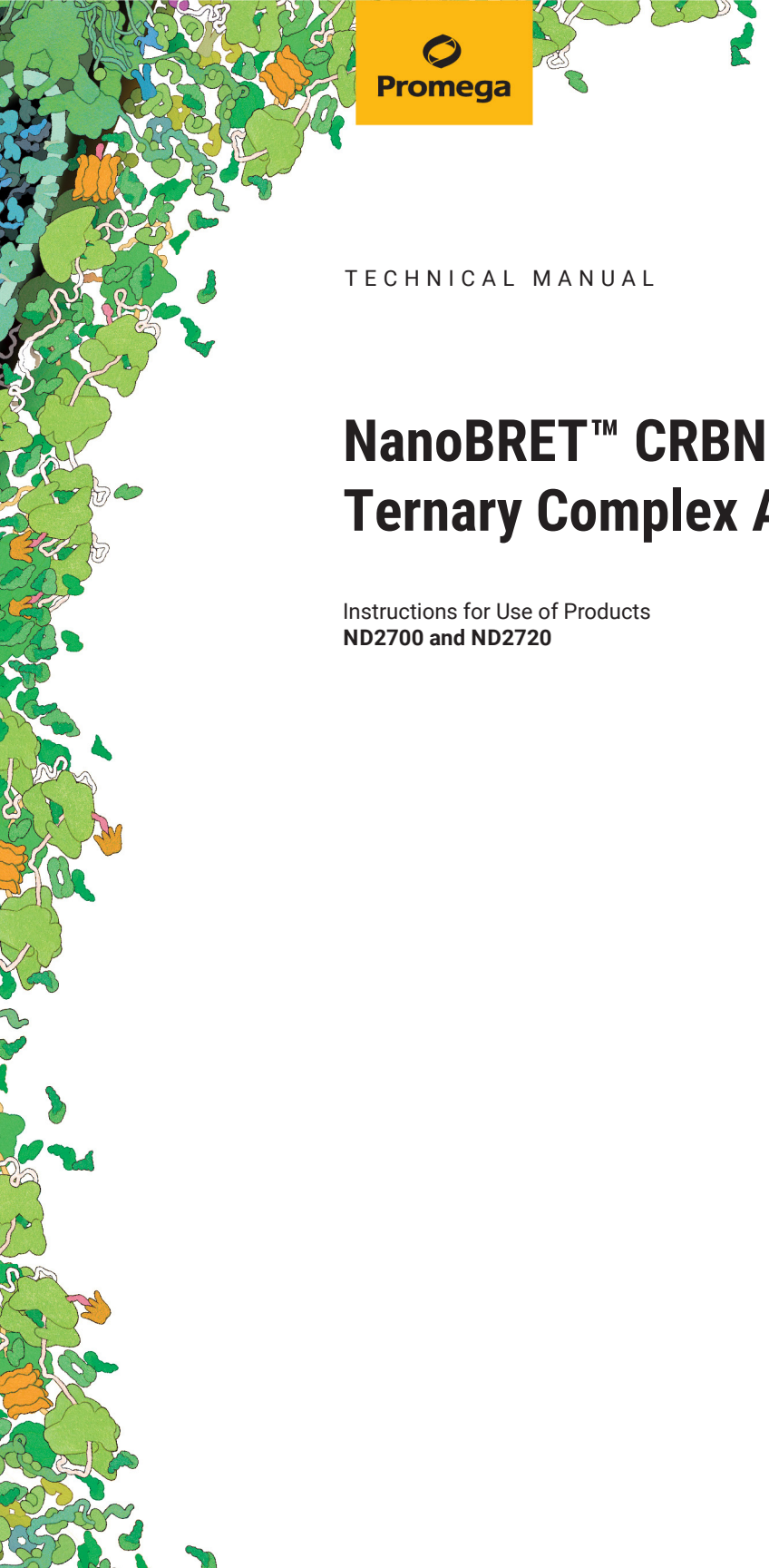

TECHNICAL MANUAL

NanoBRET™ CRBN and VHL Ternary Complex Assays

Instructions for Use of Products
ND2700 and ND2720

Revised 1/24
TM615

NanoBRET™ CRBN and VHL Ternary Complex Assays

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Targeted degradation via small molecule degraders such as a PROTAC or molecular glue require formation of a ternary complex consisting of a target protein, a degrader and E3 ligase component. Ternary complex formation is the first key mechanistic step required to achieve target ubiquitination and degradation via the ubiquitin proteasome pathway. The NanoBRET™ Ternary Complex Assays[a–h] are designed for live-cell detection of complexes that contain either VHL (von Hippel-Lindau disease tumor suppressor) or CRBN (cereblon) E3 ligase component. These assays can be read in either endpoint or kinetic formats and provide insight into how altering the degradation compound composition affects the formation, stability or both of the ternary complex (1). The NanoBRET™ Ternary Complex Assays can also be used to deconvolute degradation from other cellular pathways that lead to protein level loss and better understand the mechanism of action.

These assays use NanoBRET™ technology, a proximity-based method dependent upon energy transfer from a luminescent donor to a fluorescent acceptor that is measured using an instrument capable of reading dual-filtered luminescence (2). Given the transient nature of the ternary complex and the loss of the target protein after formation, the optimal NanoBRET™ assay configuration is with the target as the donor and the E3 ligase component as the acceptor. This means you can monitor target protein levels while simultaneously observing complex formation, made possible due to the luminescent to fluorescent ratio in the NanoBRET™ assay. Here we provide N-terminal HaloTag® fusions of VHL or CRBN, which are expressed in cells and subsequently labeled with the HaloTag® NanoBRET™ 618 Ligand to be fluorescent acceptors. As the target will be variable, we offer a suite of tools for the user to generate the appropriate luminescent donor fusion, which can be either an ectopically expressed NanoLuc® fusion or an endogenous target tagged with HiBiT using CRISPR gene editing and complemented with LgBiT. This protocol describes the optimization of the initial NanoBRET™ setup for either of these formats, including donor tag placement, expression ratio for ectopic fusions, timing and use of MG-132. Detecting the interaction requires the use of either the NanoBRET™ Nano-Glo® Detection System for endpoint detection, or the NanoBRET™ Nano-Glo® Kinetic Detection System for kinetic detection. This protocol also has optional cell viability analysis for multiplexing with the NanoBRET™ assays.

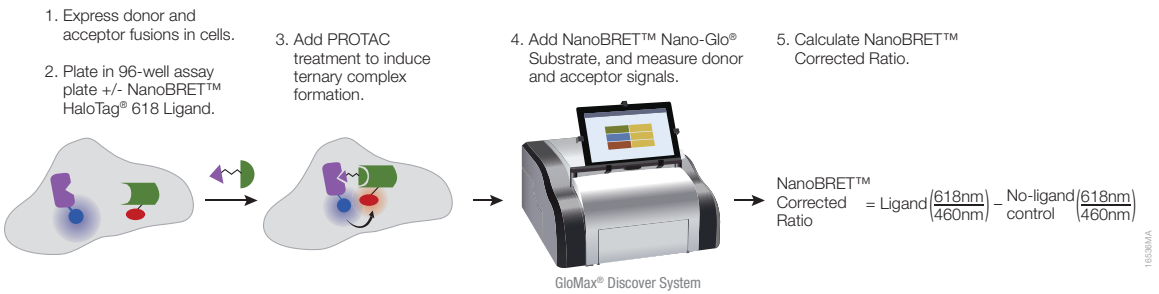

Figure 1. Overview of the five-step NanoBRET™ Ternary Complex Assay. First, HaloTag®-VHL or HaloTag®-CRBN acceptor fusion and NanoLuc® or HiBiT donor fusion are expressed in cells. Second, the cells are replated into 96-well plates, and samples are designated experimental (with HaloTag® NanoBRET™ 618 Ligand) and control (without fluorescent ligand). Third, PROTAC compounds are added to induce ternary complex formation. Fourth, the NanoBRET™ Nano-Glo® Substrate is added, and donor and acceptor signals are measured. Fifth, the corrected NanoBRET™ ratio is calculated.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT #
NanoBRET™ VHL Ternary Complex Starter Kit	**1 each**	**ND2700**

The NanoBRET™ Nano-Glo® Detection System (NanoBRET™ Nano-Glo® Substrate and HaloTag® NanoBRET™ 618 Ligand) is sufficient for approximately 200 assays in 96-well plates. NanoBRET™ VHL Ternary Complex Starter Kit includes:

- 20µg HaloTag®-VHL Fusion Vector
- 20µg pNLF1-N [CMV/Hygro] Vector
- 20µg pNLF1-C [CMV/Hygro] Vector
- 20µg HaloTag® Control Vector
- 20µg NanoLuc®-BRD4 FL Fusion Vector
- 50µl NanoBRET™ Nano-Glo® Substrate
- 20µl HaloTag® NanoBRET™ 618 Ligand

PRODUCT	SIZE	CAT #
NanoBRET™ CRBN Ternary Complex Starter Kit	**1 each**	**ND2720**

The NanoBRET™ Nano-Glo® Detection System (NanoBRET™ Nano-Glo® Substrate and HaloTag® NanoBRET™ 618 Ligand) is sufficient for approximately 200 assays in 96-well plates. NanoBRET™ CRBN Ternary Complex Starter Kit includes:

- 20µg HaloTag®-CRBN Fusion Vector
- 20µg pNLF1-N [CMV/Hygro] Vector
- 20µg pNLF1-C [CMV/Hygro] Vector
- 20µg HaloTag® Control Vector
- 20µg NanoLuc®-BRD4 FL Fusion Vector
- 50µl NanoBRET™ Nano-Glo® Substrate
- 20µl HaloTag® NanoBRET™ 618 Ligand

Storage Conditions: Store all kit components at –30°C to –10°C. The HaloTag® NanoBRET™ 618 Ligand can be frozen and thawed up to 5 times.

Note: Each starter kit contains vectors to create N- and C-terminal NanoLuc® target protein fusions using standard cloning, either the HaloTag®-CRBN or HaloTag®-VHL Fusion Vector, the positive control NanoLuc®-BRD4 FL Fusion Vector and negative control HaloTag® Control Vector.

Individual components are available to purchase separately and are listed in Section 8.D, Related Products.

Included Vector	Antibiotic Resistance Cassette
HaloTag®-VHL Fusion Vector	Kanamycin
HaloTag®-CRBN Fusion Vector	Kanamycin
HaloTag® Control Vector	Ampicillin
NanoLuc®-BRD4 FL Fusion Vector	Kanamycin

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3. Before You Begin

3.A. Assay Vector Preparation

The amount of each plasmid DNA provided with the system is sufficient for a few initial testing experiments, but we strongly advise that each plasmid be archived and propagated as transfection ready DNA. Follow standard conditions for transformation into *E. coli* for archival and propagation, and for tissue culture grade DNA preparation. For each vector, the fusion protein is constitutively expressed by a CMV promoter.

3.B. Instrument Information and Setup

To perform NanoBRET™ assays, a luminometer capable of sequentially measuring dual-wavelength windows is required. We recommend using a band pass (BP) filter for the donor signal and a long pass (LP) filter for the acceptor signal to maximize sensitivity. Filters outside of the recommended ranges will miss critical measurements and compromise data quality.

The NanoBRET™ donor emission occurs at 460nm, to measure the donor signal we recommend a band pass (BP) filter that covers close to 460nm with a band pass range of 8–80nm. For example, a 450nm/BP80 will capture the 410–490nm range.

Note: A BP filter is preferred for the donor signal measurement to selectively capture the signal peak and avoid measuring any bleed-through into the acceptor peak. You can use a short pass (SP) filter that covers the 460nm area. However, this could result in an artificially large value for the donor signal and measuring the bleed-through into the acceptor peak, which could compress the ratio calculation and reduce the assay window.

The NanoBRET™ acceptor emission occurs at 618nm, to measure the acceptor signal we recommend a long pass filter starting at 600–610nm.

Instruments capable of dual luminescence measurements are either pre-equipped with a filter selection or the filters can be purchased and added separately. For instruments using mirrors select the luminescence mirror. An integration time of 0.2–1 second is typically sufficient. Ensure that the gain or PMT is optimized to capture the highest donor signal without reaching instrument saturation.

Consult with your instrument manufacturer to determine if the proper filters are pre-installed or what steps are needed to add filters to the luminometer. For example, a special holder or cube might be required for the filters to be mounted, and the shape and thickness may vary among instruments. We have experience with the following instruments and configurations:

- The GloMax® Discover System (Cat.# GM3000) with preloaded filters for donor 450nm/8nm BP and acceptor 600nm LP. Select the preloaded BRET:NanoBRET™ 618 protocol from the Protocol menu.
- BMG Labtech CLARIOstar® with preloaded filters for donor 450nm/80nm BP and acceptor 610nm LP.
- Thermo Varioskan® with filters obtained from Edmunds Optics, donor 450nm CWL, 25mm diameter, 80nm FWHM, Interference Filter and acceptor 1 inch diameter, RG-610 Long Pass Filter.

Another popular instrument capable of measuring dual luminescence is the Perkin Elmer Envision and we recommend the following set-up:

- Mirror: Luminescence - Slot4
- Emission filter: Chroma Cat.# AT600LP - EmSlot4
- Second emission filter: Chroma Cat.# AT460/50m - EmSlot1
- Measurement height (mm): 6.5
- Measurement time (seconds): 1

3.C. NanoBRET™ Positive Control Vector

To ensure your instrument has been properly configured, we recommend testing with the NanoBRET™ Positive Control (Cat.# N1581). This vector is an artificial system that tethers the NanoLuc® and HaloTag® proteins, ensuring energy transfer. Because NanoLuc® luciferase is extremely bright and the energy transfer to the HaloTag® moiety is so efficient, the vector plasmid must be diluted with Transfection Carrier DNA to reduce its expression levels. Keep in mind that an actual protein pair is unlikely to show the same level of energy transfer efficiency and should not be compared to this artificial control. Representative data are show in Figure 2.

Note: If the NanoBRET™ Positive Control vector is to be used in the same plate as actual PPI partners, we recommend leaving an empty row of wells between PPI partners' sets and the NanoBRET™ Positive Control vector because the light from the control plasmid might cause crosstalk in adjoining wells.

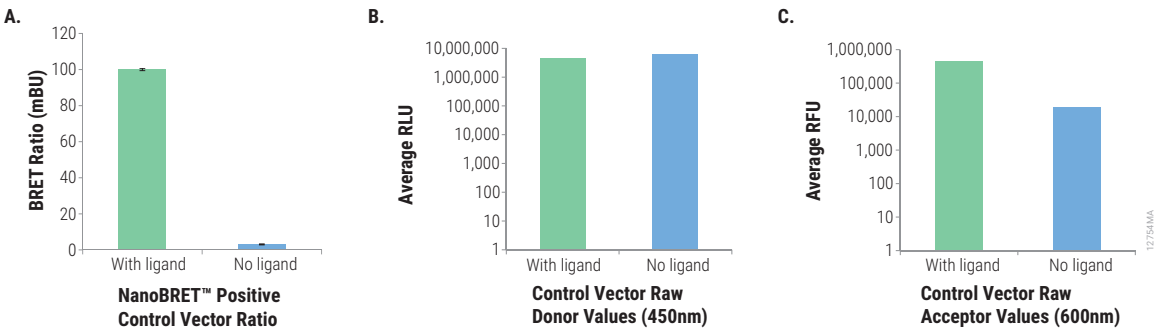

Figure 2. NanoBRET™ ratio and raw donor and acceptor measurements with the NanoBRET™ Positive Control vector. Panel A. Calculated NanoBRET™ ratio in experimental sample and no-ligand control. The no-ligand control represents the donor signal bleed-through into the acceptor channel of the NanoBRET™ ratio and should be subtracted from the experimental samples to obtain the corrected NanoBRET™ ratio. **Panel B.** Raw donor values in relative light units (RLU) measure instrument sensitivity. For most commonly used instruments, this value is typically 1,000,000 to 10,000,000 RLU for both sets of samples with or without ligand. **Panel C.** Raw acceptor values represent the energy transfer from donor to acceptor and should be higher in the experimental samples containing ligand while the no-ligand control samples represent bleed-through. Data generated using the GloMax® Discover System equipped with 450nm/8nm BP and 600nm LP filters.

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4. Assay Design Parameters and Optimization

4.A. Configuring Fusion Tags

NanoBRET™ is a proximity-based technology based on energy transfer from a luminescent donor to a fluorescent acceptor. In the NanoBRET™ Ternary Complex Assays, we have optimized the tag placement of the HaloTag® acceptor fusion, provided as an N-terminal HaloTag®-VHL or HaloTag®-CRBN fusion. To achieve an optimal NanoBRET™ signal, we recommend testing both N- and C-terminal tag placement for either the NanoLuc® or HiBiT donor, if development or functional physiology allow. To generate N- and C-terminal NanoLuc® fusions of the target protein, follow standard cloning procedures to introduce genes of interest into the NanoLuc® MCS-based fusion vectors or Flexi® Vectors as described in the individual vector protocols. For more information on generating HiBiT CRISPR insertions, visit **www.promega.com** and purchase the supporting HEK293 LgBiT Stable Cell Line (available by request through Custom Assay Services, CS1956D02).

4.B. Protein Expression Levels

Determining the protein expression level of the donor fusion relative to the acceptor fusion is important to maximize the NanoBRET™ signal and minimize background. In general, low donor fusion expression levels are recommended. This optimization step is needed for ectopically expressed NanoLuc® fusions, but not for endogenously tagged HiBiT fusions. To optimize donor expression level, we recommend performing donor dilution experiments, starting with an equal amount of donor DNA to acceptor DNA (1:1 ratio), and then diluting donor DNA to 1:10 and 1:100. Most frequently, we observe that a 1:100 donor-to-acceptor ratio is optimal for most NanoBRET™ pairs.

4.C. Test Compound Concentration and Treatment Time

Because the ternary complex only forms in the presence of a degradation compound, we recommend starting with 1µM or 10µM of the degradation compound when optimizing the assay. You can use up to 50µM of degradation compound as long as toxicity is not observed and total amount of DMSO does not exceed 0.5% by volume in the test sample.

Different target-PROTAC-E3 ligase ternary complexes may differ in formation kinetics. We recommend initially collecting measurements at multiple timepoints up to 4 hours to determine the optimal signal window.

4.D. Including Proteasome Inhibitor

To measure both protein degradation and complex formation, we recommend performing the NanoBRET™ Ternary Complex Assays in the presence and absence of proteasome inhibitor MG-132, which blocks proteasome-mediated degradation. Target protein levels are monitored as luminescence in the NanoBRET™ donor channel, and the values in the presence and absence of MG-132 treatment can be compared to determine relative amount of target degradation. In some cases, we have seen that adding MG-132 enhances the NanoBRET™ signal, producing a more more robust assay, likely due to preventing target degradation. In kinetic format, using MG-132 means you can monitor the cellular complex stability over time. We recommend testing with and without MG-132 during initial assay optimization.

Note: How the fusion protein is expressed will affect the degradation observed when comparing samples treated with or without MG-132. For example, the degradation window for an ectopically expressed NanoLuc® fusion is generally compressed relative to the degradation window of an endogenously tagged HiBiT fusion.

4.E. Controls

Two controls are described for the ternary complex assay. An unfused HaloTag® Control Vector is used as a negative control to assess interaction specificity. The positive control NanoLuc®-BRD4 FL Fusion Vector is used with commercially available known PROTAC compounds MZ1 for the VHL:BRD4 interaction (3), and dBET6 for the CRBN:BRD4 interaction (4). The HaloTag® Control Vector is designed for use when optimizing a new assay pair. The NanoLuc® BRD4 FL Fusion Vector can be used as an assay plate control during new assay optimization or during experimental testing of an optimized assay.

5. NanoBRET™ Ternary Complex Assay Protocol

Materials to Be Supplied By the User

- HEK293 or similar mammalian cells
- white, 96-well plate (Costar® Cat.# 3917) or 384 well plate (Corning® Cat.# 3570)
- tissue culture equipment and reagents (see Section 8.B, Composition of Buffers and Solutions section).
- DPBS (GIBCO Cat.# 14190-144)
- 0.05% Trypsin/EDTA (GIBCO™ Cat.# 25300-054)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- DMEM (GIBCO™ Cat.#11995-065)
- fetal bovine serum (Seradigm Cat.# 89510-194)
- Opti-MEM™ I Reduced Serum Medium, no phenol red (Thermo Fisher Scientific Cat.# 11058021)
- DMSO (Sigma Cat.# 2650)
- Nuclease-Free Water (Cat.# P1191)
- **Optional:** BRD4/CRBN control assay compound dBET6 (Selleckchem Cat.# S8762) **or** BRD4/VHL control assay compound MZ1 (Tocris Cat.# 6154) **or** ARV-771 (MedChemExpress Cat.# HY-100972)
- user-defined target PROTAC test compounds
- MG-132 proteasome inhibitor (Selleckchem Cat.# S2619)
- luminometer capable of measuring dual-filtered luminescence (e.g., GloMax® Discover System Cat.# GM3000; see Section 3.B for more information)

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5.A. Transfection Protocols for NanoBRET™ Ternary Complex Assays

The following transient transfection conditions are for mammalian HEK293 cells. Other cells lines may require optimization. If using a transfection reagent other than FuGENE® HD Transfection Reagent, follow the manufacturer's recommendations but keep the same relative donor-to-acceptor DNA ratio.

Note: Follow the appropriate transfection protocol, as DNA amounts required may differ for the assay control target versus a user-defined target. The four different transfection schemes are:

- Checking the Instrument Setup with the NanoBRET™ Positive Control
- Checking the NanoBRET™ Ternary Complex Assay performance with the NanoLuc®-BRD4 Fusion Vector and HaloTag®-VHL **or** HaloTag®-CRBN **or** HaloTag® Control Vector
- Assay optimization: User-generated NanoLuc® target fusion vectors transfected with HaloTag®-VHL or HaloTag®-CRBN Fusion Vector
- Transfecting the HaloTag®-VHL or HaloTag®-CRBN Fusion Vector into cells with an endogenously tagged HiBiT CRISPR fusion protein

Following the transfection step, all the remaining steps in the protocol remain identical regardless of type of transfection performed.

Optional: Transfection Conditions for the NanoBRET™ Positive Control to Check Instrument Setup

1. Culture HEK293 cells appropriately prior to assay.
2. Remove medium from cell flask via aspiration, trypsinize and allow cells to dissociate from the flask bottom.
3. Neutralize trypsin using cell culture medium, count cells to estimate density and resuspend to a final cell density to 4×10^5/ml in cell culture medium.
4. Plate 2ml of cells (800,000 cells) into each well of a six-well plate.
5. Allow the cells to attach and recover for 4–6 hours at 37°C, 5% CO_2.
6. Prepare a transfection mixture consisting of 2µg Transfection Carrier DNA + 0.002µg NanoBRET™ Positive Control vector diluted in water.
7. Add 100µl of Opti-MEM™ I Reduced Serum Medium, no phenol red, to the transfection mixture, and mix well.
8. Add 6µl of FuGENE® HD Transfection Reagent, and incubate at room temperature for 10 minutes.
9. Dispense transfection mixture to wells with attached cells and express proteins for approximately 20 hours at 37°C, 5% CO_2.
10. Proceed to Section 5.B.

Transfection Conditions to Check the NanoBRET™ Ternary Complex Assay Performance

1. Culture HEK293 cells appropriately prior to assay.
2. Remove medium from cell flask via aspiration, trypsinize and allow cells to dissociate from the flask bottom.
3. Neutralize trypsin using cell culture medium, count cells to estimate density, and resuspend to a final cell density to 4×10^5/ml in cell culture medium.
4. Plate 2ml of cells (800,000 cells) into each well of a six-well plate.
5. Allow the cells to attach and recover for 4–6 hours at 37°C, 5% CO_2.
6. Prepare a 1:100 transfection mixture consisting of 2µg HaloTag®-CRBN **or** HaloTag®-VHL **or** HaloTag Control Vector + 0.02µg NanoLuc®-BRD4 Fusion Vector diluted in water.
7. Add 100µl of Opti-MEM™ I Reduced Serum Medium, no phenol red, to the transfection mixture, and mix well.
8. Add 6µl of FuGENE® HD Transfection Reagent, and incubate at room temperature for 10 minutes.
9. Dispense transfection mixture to wells with attached cells and express proteins for approximately 20 hours at 37°C, 5% CO_2.
10. Proceed to Section 5.B.

Transfection Conditions for Optimizing Donor Tag Placement and Donor-to-Acceptor Ratio

1. Culture HEK293 cells appropriately prior to assay.
2. Remove medium from cell flask by aspiration, trypsinize and allow cells to dissociate from the flask bottom.
3. Neutralize trypsin using cell culture medium, count cells to estimate density and resuspend to a final density of 4×10^5 cells/ml in cell culture medium.
4. Plate 2ml of cells (800,000 cells) into a well of a six-well plate sufficient for the number of planned assays. After transfection and cell division, three wells of a six-well plate yield enough cells for assaying one 96-well plate. For larger-scale experiments, transfect cells in T flasks or dishes, scaling the quantity of transfection materials accordingly.
5. Allow the cells to attach and recover for 4–6 hours at 37°C, 5% CO_2.
6. Prepare a transfection mixture consisting of DNA amounts according to the table below. Clone both N- and C-terminal NanoLuc® target protein fusions and test the fusion proteins to find the orientation that provides the best assay window.

5.A. Transfection Protocols for NanoBRET™ Ternary Complex Assays (continued)

Desired Ratio	HaloTag® Vector (Acceptor)	Amount of N- or C- Terminal NanoLuc® Fusion Vector Diluted in Water (Donor)
1:1 (NanoLuc to HaloTag)	1µg HaloTag®-CRBN or HaloTag®-VHL Fusion Vector or HaloTag® Control Vector	1µg NanoLuc® Fusion Vector
1:10 (NanoLuc to HaloTag)	2µg HaloTag®-CRBN or HaloTag®-VHL Fusion Vector or HaloTag® Control Vector	0.2µg NanoLuc® Fusion Vector
1:100 (NanoLuc to HaloTag)		0.02µg NanoLuc® Fusion Vector

7. Add 100µl of Opti-MEM™ I Reduced Serum Medium, no phenol red, to the transfection mixture, and mix well.
8. Add 6µl of FuGENE® HD Transfection Reagent and incubate at room temperature for 10 minutes.
9. Add transfection mixture to wells with attached cells, and express overnight (18–24 hours) at 37°C, 5% CO_2.
10. Proceed to Section 5.B.

Transfection Conditions for Endogenously Tagged HiBiT CRISPR Fusion Protein

Note: Cell lines which contain a HiBiT CRISPR fusion in a cell line stably expressing LgBiT (such as HEK293 LgBiT Stable Cells, Cat.# N2672) do not require additional LgBiT expression. If the cell line being tested contains an endogenous HiBiT CRISPR fusion only, LgBiT must be expressed by transfecting the CMV LgBiT Vector (Cat.# N2681).

1. Culture the cell line with endogenous target HiBiT knock-in appropriately prior to assay.
2. Remove medium from cell flask via aspiration, trypsinize, and allow cells to dissociate from the flask.
3. Neutralize trypsin using cell culture medium, count to estimate density, and resuspend to a final density of 4×10^5 cells/ml in cell culture medium.
4. Plate 2ml of cells (800,000 cells) into each well of a six-well plate sufficient for the number of planned assays. After transfection and cell division, three wells of a six-well plate yield enough cells for one 96-well plate. For larger scale experiments, transfect cells in T flasks or dishes, scaling the quantity of transfection materials accordingly.
5. Allow the cells to attach and recover for 4–6 hours at 37°C, 5% CO_2.
6. When ready to transfect, prepare a transfection mixture appropriate to your donor expression format, based on the table below:

Donor Expression Format	Desired Ratio	HaloTag® Vector	CMV LgBiT Vector
Endogenous HiBiT Fusion	1:1 (LgBiT to HaloTag)	1µg HaloTag®-VHL or HaloTag®-CRBN Fusion Vector or HaloTag® Control Vector	1µg LgBiT Vector
	1:10 (LgBiT to HaloTag)	2µg HaloTag®-VHL or HaloTag®-CRBN Fusion Vector or HaloTag® Control Vector	0.2µg LgBiT Vector
	1:100 (LgBiT to HaloTag)		0.02µg LgBiT Vector
HiBiT CRISPR Fusion Stably Expressing LgBiT	N/A	2µg HaloTag®-VHL or HaloTag®-CRBN Fusion Vector or HaloTag® Control Vector	N/A

7. Add 100µl of Opti-MEM™ I Reduced Serum Medium, no phenol red, to the transfection mixture, and mix well.
8. Add 6µl of FuGENE® HD Transfection Reagent, and incubate at room temperature for 10 minutes.
9. Dispense transfection mixture to wells with attached cells and express proteins for approximately 20 hours at 37°C, 5% CO_2.
10. Proceed to Section 5.B.

5.B. Replating Transfected HEK293 Cells into Multiwell Plates and Adding HaloTag® NanoBRET™ 618 Ligand

1. For each well in a six-well plate, remove medium from cells, and wash with 1ml of DPBS. Discard.
2. Add 0.5ml of 0.05% trypsin-EDTA and incubate at room temperature until cells lift from well bottom.
3. Add 2ml of cell culture medium to neutralize trypsin, mix to collect and resuspend cells, and transfer cell suspension to a 15ml conical tube.
4. Spin cells down at 125 × *g* for 5 minutes. Discard cell culture medium and resuspend in an equal volume of assay medium (Opti-MEM™ I Reduced Serum Medium, no phenol red + 4% FBS).
5. Count to estimate cell density and adjust density to 2.5×10^5 cells/ml in assay medium. To cover an entire 96-well plate, you need at least 8ml of cells at this concentration. For a 384-well plate, you need approximately 13ml of cells at this concentration.
6. Divide cells into two pools, and add HaloTag® NanoBRET™ 618 Ligand or DMSO vehicle as follows:

   Experimental samples (+ ligand): Add 1µl of 0.1mM HaloTag® NanoBRET™ 618 Ligand per milliliter of cells (100nM final concentration).

   No-acceptor controls (– ligand): Add 1µl of DMSO per milliliter of cells (0.1% DMSO final concentration).

5.B. Replating Transfected HEK293 Cells into Multiwell Plates and Adding HaloTag® NanoBRET™ 618 Ligand (continued)

7. Plate cells in the volumes indicated below:

   96-well format: Dispense 80µl of each pool of the cells prepared in Step 6 in at least 3–4 wells.

   384-well format with endpoint detection: Dispense 32µl of each pool of the cells prepared in Step 6 in at least 3–4 wells.

   384-well format with kinetic detection: Dispense 36µl of each pool of the cells prepared in Step 6 in at least 3–4 wells.

8. Incubate plates at 37°C, 5% CO_2 overnight (18–24 hours).

9. Proceed to Section 5.C.

5.C. Adding MG-132 and Test Compounds, and Detecting Ternary Complex Formation

Determine which detection protocol to follow. Choose between measuring the endpoint or assessing kinetic changes in live cells.

Notes:

a. The ternary complex positive controls can be performed with or without proteasome inhibitor for endpoint detection. When performing kinetic monitoring of the ternary complex, we recommend pretreating with proteasome inhibitor for the ternary assay positive control. Samples without proteasome inhibitor may be included to monitor degradation. If MG-132 is deemed unnecessary upon completion of assay optimization, cell plating volumes can be adjusted in Section 5.B to account for volume differences. For example, instead of adding 10µl of MG-132 to each well of a 96-well plate, add an additional 10µl of cells for a total of 90µl.

b. **Regarding Dose-Response Curves (DRC):** If compounds are to be tested at a range of concentrations, perform serial dilutions in diluent containing the same amount of solvent as the highest concentration. For example, if the highest 10X concentration contains 1% DMSO, subsequent dilutions should be done in assay media containing 1% DMSO to keep the final concentration at 0.1% DMSO for all samples. For a vehicle or zero control, add DMSO containing media without compound. The NanoBRET™ assay has been tested at up to 0.5% final DMSO concentration with no consequence. Higher DMSO concentrations may be tolerated in the assay.

c. For samples pretreated with MG-132, perform Steps 1–3. For samples **not** treated with MG-132, skip Steps 1 and 2 and proceed directly to Step 3.

Live-Cell Endpoint Detection using NanoBRET™ Nano-Glo Detection System

1. Prepare a 9X solution of MG-132 proteasome inhibitor (90µM) in Opti-MEM™ I Reduced Serum Medium, no phenol red. Prepare a 9X concentration of DMSO to use as a negative control by adding an equivalent amount of DMSO to Opti-MEM™ I Reduced Serum Medium, no phenol red.

   96-well format: Add 10µl of MG-132 or DMSO solution to each well for a final concentration of 10µM.

   384-well format: Add 4µl of MG-132 or DMSO solution to each well for a final concentration of 10µM.

2. Incubate plates at 37°C, 5% CO_2 for 30 minutes.

3. Prepare a 10X concentration of test compound in Opti-MEM™ I Reduced Serum Medium, no phenol red (e.g., 10µM for a 1µM final concentration). Prepare a 10X concentration of DMSO to use as a negative control by adding an equivalent amount of DMSO to Opti-MEM™ I Reduced Serum Medium, no phenol red.

   96-well format: Add 10µl of test compound or DMSO solution to each well.

   384-well format: Add 4µl of test compound or DMSO solution to each well.

   Note: For the VHL/BRD4 control assay, treat with 1µM MZ1 for 2 hours. For the CRBN/BRD4 control assay, treat with 1µM dBET6 for 2 hours.

4. Incubate plates at 37°C, 5% CO_2 for 1–4 hours.

5. Prepare a 5X solution of NanoBRET™ Nano-Glo® Substrate in Opti-MEM™ I Reduced Serum Medium, no phenol red. This is a 100-fold dilution of the stock reagent. For one 96-well plate, prepare a minimum of 2.5ml of medium + 25µl of stock reagent. For one 384-well plate, prepare a minimum of 4ml of medium + 40µl of stock reagent. Substrate volume provided in the kit allows for the preparation of 4% extra detection solution.

   Note: Use the 5X solution within 2 hours if stored at room temperature or within 4 hours if stored at 4°C.

6. Add substrate to cells and shake plate to mix for 30 seconds. We recommend using an electromagnetic mixer for the 384-well format.

   96-well format: Add 25µl of substrate.

   384-well format: Add 10µl of substrate.

7. Measure donor emission (460nm) and acceptor emission (618nm) within 10 minutes of substrate addition using a NanoBRET™ Assay-compatible luminometer (see instrument requirements in Section 3.B).

8. Proceed to Section 5.D for NanoBRET™ calculations.

5.C. Adding MG-132 and Test Compounds, and Detecting Ternary Complex Formation (continued)

Live-Cell Kinetic Detection using NanoBRET™ Nano-Glo® Kinetic Detection System

1. For samples treated with MG-132, prepare substrate as follows. Then proceed to Step 3.

   96-well format: Prepare a 1X solution of Nano-Glo® Vivazine™ substrate (a 1:100 dilution of the stock reagent) plus 10µM MG-132 proteasome inhibitor in Opti-MEM™ I Reduced Serum Medium, no phenol red + 4% FBS.

   384-well format: Prepare a 2X solution of Nano-Glo® Vivazine™ substrate (a 1:50 dilution of the stock reagent) plus 20µM MG-132 proteasome inhibitor in Opti-MEM™ I Reduced Serum Medium, no phenol red + 4% FBS.

2. For samples **not** treated with MG-132, prepare substrate as follows. Then proceed to Step 3.

   96-well format: Prepare a 1X solution of Nano-Glo® Vivazine™ substrate (a 1:100 dilution of the stock reagent) in Opti-MEM™ I Reduced Serum Medium, no phenol red + 4% FBS.

   384-well format: Prepare a 2X solution of Nano-Glo® Vivazine™ substrate (a 1:50 dilution of the stock reagent) in Opti-MEM™ I Reduced Serum Medium, no phenol red + 4% FBS.

3. Add Vivazine™ solution to each well.

   96-well format: Aspirate medium and dispense 90µl of 1X Vivazine™ solution.

   384-well format: Dispense 36µl of 2X Vivazine™ solution to 36µl of cells.

4. Incubate plate for 30–60 minutes at 37°C, 5% CO_2 to equilibrate substrate luminescence and treat with MG-132, if applicable.

5. Prepare a 10X concentration of test compound in Opti-MEM™ I Reduced Serum Medium, no phenol red (e.g., 10µM for a 1µM final concentration). Prepare a 10X concentration of DMSO to use as a negative control by adding an equivalent amount of DMSO to Opti-MEM™ I Reduced Serum Medium, no phenol red.

   96-well format: Add 10µl of test compound or DMSO solution to each well.

   384-well format: Add 8µl of test compound or DMSO solution to each well.

   Note: For the VHL/BRD4 control assay, treat with 1µM MZ1. For the CRBN/BRD4 control assay, treat with 1µM dBET6.

6. Immediately collect kinetic measurements of donor emission (460nm) and acceptor emission (618nm) every 3–5 minutes up to 6 hours after adding test compound using a NanoBRET™ Assay-compatible luminometer (see instrument requirements in Section 3.B).

7. Proceed to Section 5.D for NanoBRET™ calculations.

5.D. NanoBRET™ Calculations

1. Divide the acceptor emission value (e.g., 618nm) by the donor emission value (e.g., 460nm) for each sample to generate raw NanoBRET™ ratio values:

   $$\frac{618nm_{Em}}{460nm_{Em}} = \text{Raw NanoBRET™ Ratio} = BU$$

2. To convert raw NanoBRET™ units (typically decimal values) to milliBRET units (mBU; whole numbers), multiply each raw BRET value by 1,000.

   $$\frac{618nm_{Em}}{460nm_{Em}} = BU \times 1{,}000 = mBU$$

3. Determine the mean NanoBRET™ ratio for each set of samples: Experimental samples with HaloTag® NanoBRET™ 618 Ligand and no-ligand control samples. To factor in donor-contributed background or bleedthrough, subtract the no-ligand control mean from the Experimental mean for the corrected NanoBRET™ ratio.

   Mean mBU experimental – Mean mBU no-ligand control = Mean corrected mBU

4. **Optional:** Z´ and Z factor calculations can be generated to gauge assay consistency. The Z´ factor estimates assay consistency by comparing the mean and standard deviation (STDV) values of the experimental samples and the no-ligand control samples.

   $$\text{Z´ factor} = 1 - \left[\frac{(3X\ STDV\ experimental + 3X\ STDV\ no\text{-}ligand\ control)}{(Mean\ mBU\ experimental - Mean\ mBU\ no\text{-}ligand\ control)}\right]$$

In the presence of a degradation compound, a Z factor (different from a Z´ factor) takes into account both the assay variability and the difference between a treated sample and a vehicle control (delta). Use corrected mBU and STDV for these calculations. In general, an assay with a Z´ or Z value between 0.5–1 is considered to be robust with lower assay variability. Treated samples refer to samples treated with degradation compound. For the NanoBRET™ Ternary Complex assays, an increase in mBU is expected for treated samples relative to untreated samples.

$$\text{Z factor} = 1 - \left[\frac{(3X\ STDV\ untreated + 3X\ STDV\ treated)}{(Mean\ mBU\ treated - Mean\ mBU\ untreated)}\right]$$

6. Representative Data

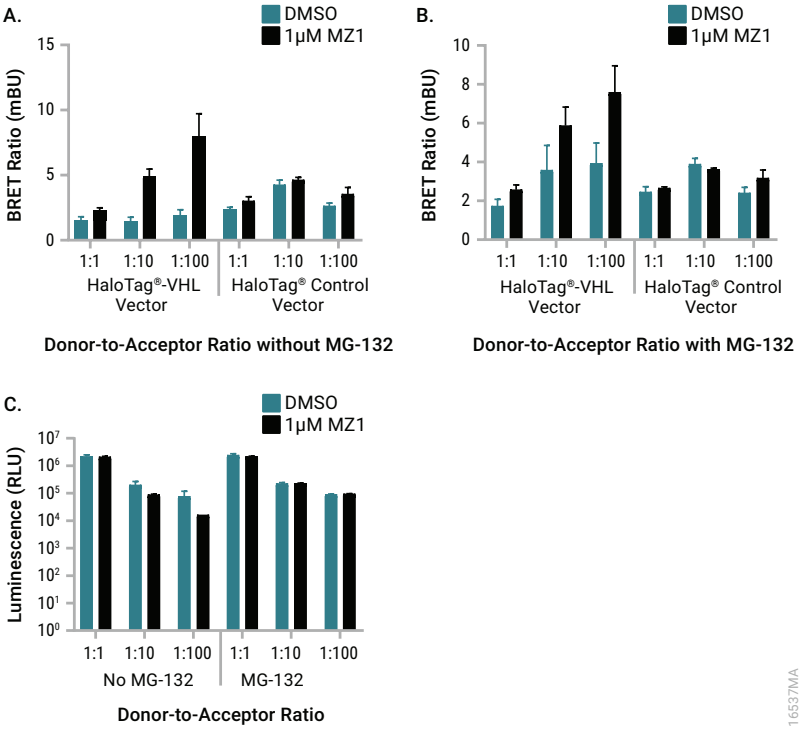

Figure 3. NanoBRET™ Ternary Complex Assay using the HaloTag®-VHL and NanoLuc®-BRD4 fusion proteins. Panel A. Assay optimization in the absence of MG-132 proteasome inhibitor. Donor-to-acceptor ratios of 1:1, 1:10 and 1:100 were tested with both the specific HaloTag®-VHL Fusion Vector and HaloTag® Control Vector. Samples were treated with 1µM MZ1 PROTAC compound or DMSO control for 2 hours and NanoBRET™ signal was measured on a GloMax® Discover System instrument using the NanoBRET™ Nano-Glo® Detection System. A specific increase in NanoBRET™ signal was observed for BRD4/VHL fusion proteins with MZ1 treatment in the absence of MG-132 proteasome inhibitor when compared to the HaloTag® protein control. **Panel B.** Assay optimization in the presence of MG-132 proteasome inhibitor. Donor-to-acceptor ratios of 1:1, 1:10 and 1:100 were tested with both the specific HaloTag®-VHL Fusion Vector and HaloTag® Control Vector. Samples were pretreated with 10µM MG-132 for 30 minutes, then treated with 1µM MZ1 PROTAC compound or DMSO control for 2 hours. NanoBRET™ signal was measured on a GloMax® Discover System instrument using the NanoBRET™ Nano-Glo® Detection System. A specific increase in NanoBRET™ signal was observed for BRD4/VHL with MZ1 treatment in the presence of MG-132 proteasome inhibitor when compared to the HaloTag® protein control. **Panel C.** NanoBRET™ donor luminescence values for NanoLuc®-BRD4 fusion protein at 1:1, 1:10 and 1:100 donor-to-acceptor ratios with and without MG-132 pretreatment, and with and without 1µM MZ1 PROTAC compound treatment. The absence of MG-132 resulted in an expected decrease in donor luminescence with MZ1 treatment, and donor signal stabilized in the presence of MG-132.

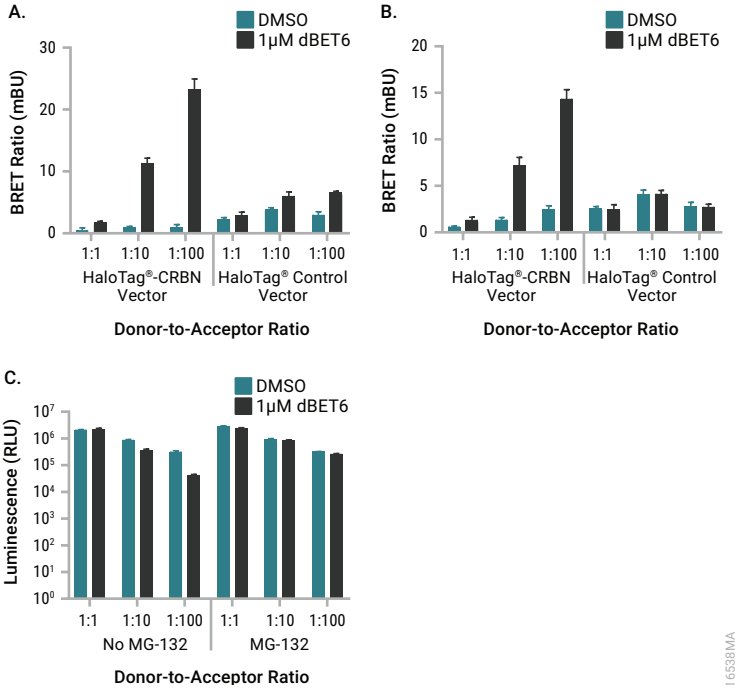

Figure 4. NanoBRET™ Ternary Complex Assay using the HaloTag®-CRBN and NanoLuc®-BRD4 fusion proteins. Panel A. Assay optimization in the absence of MG-132 proteasome inhibitor. Donor-to-acceptor ratios of 1:1, 1:10 and 1:100 were tested with both the specific HaloTag®-CRBN fusion vector and HaloTag® Control Vector. Samples were treated with 1µM dBET6 PROTAC compound or DMSO control for 2 hours and NanoBRET™ signal was measured on a GloMax® Discover System using the NanoBRET™ Nano-Glo® Detection System. A specific increase in NanoBRET™ signal was observed for BRD4/CRBN with dBET6 treatment in the absence of MG-132 proteasome inhibitor when compared to the HaloTag® protein control. **Panel B.** Assay optimization in the presence of MG-132 proteasome inhibitor. Donor-to-acceptor ratios of 1:1, 1:10 and 1:100 were tested with both the specific HaloTag®-CRBN Fusion Vector and HaloTag® Control Vector. Samples were pretreated with 10µM MG-132 for 30 minutes, then treated with 1µM dBET6 PROTAC compound or DMSO control for 2 hours. NanoBRET™ signal was measured on a GloMax® Discover System using the NanoBRET™ Nano-Glo® Detection System. A specific increase in NanoBRET™ signal is observed for BRD4/CRBN with dBET6 treatment in the presence of MG-132 proteasome inhibitor when compared to the unfused HaloTag control. **Panel C.** NanoBRET™ donor luminescence values for NanoLuc®-BRD4 fusion protein at 1:1, 1:10 and 1:100 donor-to-acceptor ratios with and without MG-132 pretreatment and with and without 1µM dBET6 PROTAC compound treatment. The absence of MG-132 resulted in an expected decrease in donor luminescence with dBET6 treatment, and donor signal stabilized in the presence of MG-132.

Note: With highly potent degradation compounds, you may observe an increase in NanoBRET™ ratio for treated samples even with the HaloTag® Control Vector and in the absence of MG-132. This increase is typically minor compared to the specific induced interaction with the HaloTag®-VHL or HaloTag®-CRBN Fusion Vectors. Testing in the presence of MG-132 will stabilize the luminescent donor and decrease or eliminate the increase in NanoBRET™ ratio observed with the HaloTag® protein control.

6. Representative Data (continued)

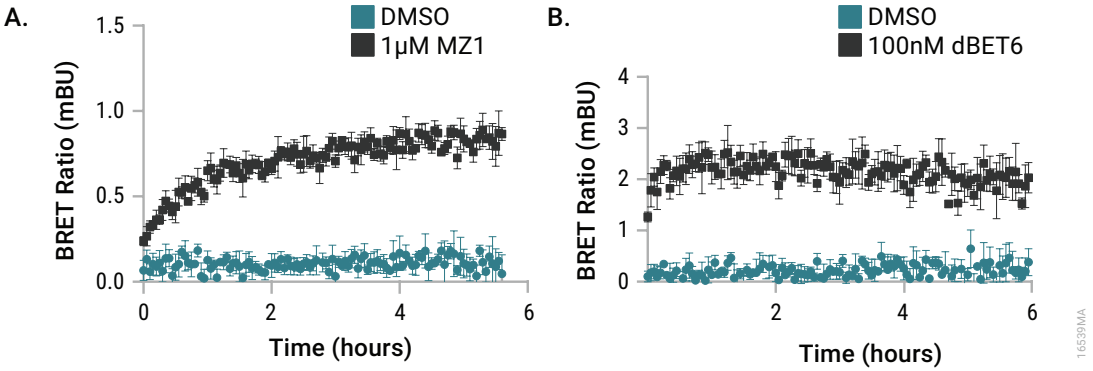

Figure 5. Measurements taken with NanoBRET™ Ternary Complex Assay in kinetic format. Panel A. The HaloTag®-VHL Fusion Vector was transiently transfected into cells stably expressing LgBiT protein and an endogenously tagged HiBiT-BRD4 CRISPR fusion. Cells were treated with 1µM MZ1 or DMSO and the NanoBRET™ signal was measured continuously for 5.5 hours using the NanoBRET™ Kinetic Detection System. Data were collected using a GloMax® Discover System. **Panel B.** The HaloTag®-CRBN Fusion Vector was transiently transfected into cells stably expressing LgBiT protein and an endogenously tagged HiBiT-BRD4 CRISPR fusion. Cells were treated with 100nM dBET6 or DMSO and the NanoBRET™ signal was measured continuously for 6 hours using the NanoBRET™ Kinetic Detection System. Data were collected using a GloMax® Discover System.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
No NanoBRET™ ratio even with the NanoBRET™ Positive Control Assay	Improper instrument setup. • Make sure luminometer has the proper filters: 460nm/8–80nm BP for donor signal; 600–610nm LP for acceptor signal • Make sure PMT or gain is set to detect donor signal without instrument saturation.
	Lack of expression of protein partners. Check expression of HiBiT or NanoLuc® fusions by luminescence or 460nm reading. Check expression of fluorescent HaloTag® fusion by cell-to-gel and band quantification in a fluoroimager. See the *HaloTag® Mammalian Pull-Down and Labeling Systems Technical Manual* #TM342 for more information.
	Donor and acceptor tags are not within proximity for energy transfer to occur. Test both N- and C-terminal tagging of HiBiT or NanoLuc® donor fusions.
	Lack of LgBiT protein expression when using HiBiT CRISPR cell lines. Check LgBiT expression by adding HiBiT Control Protein (Cat.# N3010) in the Nano-Glo® HiBiT Lytic Detection System (Cat.# N3030).
	Improper relative amounts of HaloTag® and NanoLuc® vectors. Follow the recommended ratios for transfecting HaloTag® and NanoLuc® vectors.
	Improper calculations. Divide the acceptor value by the donor value (618nm ÷ 460nm). Optionally multiply by 1,000 to convert to mBU. To account for background contribution, subtract the ratio of the no-ligand control from the ratio of the experimental samples.

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7. Troubleshooting (continued)

Symptoms	Causes and Comments
Poor Z´ and Z factor values	High variability in numbers. • Ideally a robust assay has Z´ values of 0.5–1. Consider dispensing by automation to reduce variability. Z´ values could be lower in the 384 well format. • A Z factor value takes into account both the assay variability as well as the degree of effect of a modulator such as an inhibitor. A weak degradation compound will produce a small change (delta) between treated and untreated samples, resulting in a suboptimal Z factor value not due to the assay consistency.
	NanoLuc® signal is close to the instrument limit of detection. The recommended amount of donor DNA in the control assay has been optimized for detection on most commonly used instruments. If the luminometer being used has lower sensitivity, increase the amount of NanoLuc® donor DNA. Do not exceed a 1:1 ratio between NanoLuc® donor and HaloTag® acceptor DNAs.
	Signal from a HiBiT CRISPR cell line is close to instrument limit of detection • Confirm that cell line being used is endogenously expressing HiBiT tagged protein. • Confirm that LgBiT protein is expressed within the cell by adding the HiBiT Control Protein (Cat.# N3010) in the Nano-Glo® HiBiT Lytic Detection System (Cat.# N3030).
Ratios and raw values are different from those shown on examples	The absolute raw values and ratios may vary among instruments and set ups. Confirm the proper biological response (increase in signal) is observed such as the induction of interaction between BRD4 and VHL by MZ1, or between BRD4 and CRBN by dBET6.
	The absolute raw values and ratios will vary among PPI systems. Absolute NanoBRET™ values are dependent on the proximity of the protein partners, the kD of the interaction, the relative occupancy with other interacting proteins, and the instrument set up. When possible check specificity with a degradation compound known to induce ternary complex formation.
Unable to express proteins	Suboptimal transfection conditions. Follow the recommended strategy for determining optimal relative amounts of HaloTag® and NanoLuc® fusion vectors.

Symptoms	Causes and Comments
Unable to detect increase in signal when using a known degradation compound	Poor compound potency or permeability. Test a series of with compound concentrations to determine optimal treatment concentration. For example, using 0.1–10µM of compound.
	Ternary complex formation, stability or both is time dependent. Test multiple timepoints, or perform the assay in kinetic format to determine optimal time of detection. Ternary complex formation usually occurs in 1–2 hours.
	Donor expression is too high when using transient transfection. Reduce the relative level of donor expression or test target as a HiBiT CRISPR knock-in if possible.
	Donor expression is too low. If endogeneously expressing a HiBiT fusion, test by transfecting a NanoLuc® fusion of the target protein. If using kinetic detection, first test and optimize tag orientation and protein expression levels in endpoint detection format.
	Compound concentration too low. Assay sensitivity can be decreased if compound treatment concentration is too low. Even with potent degradation compounds, we recommend treating with 0.1–10µM.
Can detect ternary complex formation but unable to detect HiBiT or NanoLuc® target degradation in the absence of MG-132	Ectopic expression of NanoLuc® fusion donor. Reduce the relative level of NanoLuc® fusion expression because overexpression may mask degradation.
	Forming the ternary complex may not result in target degradation. This assay can separately detect ternary complex formation and target degradation. The ternary complex can form without resulting ubiquitination or proteasomal degradation or both.
	Timeframe of target signal measurement was not sufficient to detect degradation. Compound may show degradation at timepoints longer than ternary complex formation.
Measure a decrease in NanoLuc® or HiBiT donor luminescence is observed without detecting ternary complex formation	Poor cell health or compound toxicity. Ensure cells are still viable at the time of assay measurement by multiplexing with the CellTiter-Glo® 2.0 Assay.
No difference in ternary complex formation observed when comparing ± MG-132 treatment	Adding MG-132 may or may not increase ternary complex assay signal. We suggest testing ± MG-132 as a means to obtain optimal signal window. However, MG-132 treatment may have no effect on signal window.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Cannot detect NanoBRET™ ratio using other HaloTag® fluorescent ligands	The optimal fluorescent ligand for HaloTag is the NanoBRET™ 618 Ligand. Do not use other HaloTag® fluorescent ligands. The signal will be reduced or absent.
Unequal amounts of detection reagents	There is enough material provided for each of the detection reagent components for the number of assays. Because we recommend including a set of samples without HaloTag® NanoBRET™ 618 Ligand as a negative control, you may end up with extra ligand. The individual detection reagents are also available to purchase separately.
Discolored HaloTag® NanoBRET™ 618 Ligand	The HaloTag® NanoBRET™ 618 Ligand is typically a hue of pink to red color but there might be instances where it appears a lighter hue or colorless. This is due to varying degrees of molecular closeness. In the close form the ligand is colorless. When added to medium, the ligand converts completely to the open usable form. To confirm chemical integrity, dilute 1µl of ligand in 1ml of Opti-MEM™ I Reduced Serum Medium, no phenol red, and check fluorescence by exciting at 593nm±4nm and measuring the emission at 621nm±4nm. For the GloMax®-Multi Detection System, use the green channel (Ex: 525nm, Em: 580–640nm).

8. Appendix

8.A. Multiplexing with the CellTiter-Glo® 2.0 Assay

In some cases, you may want to determine the cell viability or compound toxicity or both plus perform the NanoBRET™ assay. Multiplexing with another assay will give you more data from a single well. Assess cell health using the ready-to-use CellTiter-Glo® 2.0 Assay, a luminescent assay that quantitates the amount of ATP present, which indicates the presence of metabolically active cells. Figure 11 shows example data.

1. Equilibrate CellTiter-Glo® 2.0 reagent to room temperature.
2. Following NanoBRET measurements, add an equal volume of CellTiter-Glo® 2.0 Reagent per well and mix on a plate shaker at 500–700 rpm for 5 minutes. For example, add 125µl of CellTiter-Glo® 2.0 Reagent to a 96-well plate.

   Note: Total well volume in a 96-well plate should not exceed 250µl after adding Cell Titer-Glo® 2.0 Reagent. If multiplexing the CellTiter-Glo® 2.0 Assay with the NanoBRET™ assay where fusion proteins were transiently transfected, add a higher concentration of compound and Nano-Glo® substrate to maintain the 125µl well volume for the NanoBRET™ assay.
3. Incubate the plate at room temperature for 30 minutes to lyse cells and quench the NanoLuc® signal.
4. After the 30-minute incubation is complete, measure total luminescence on a luminometer. If using the GloMax® Discover System, select the CellTiter-Glo® protocol.

5. If determining compound toxicity, compare the luminescence (RLU) of vehicle-containing samples versus compound-containing samples. Keep in mind that even if some toxicity is observed, the NanoBRET ratio is only derived from the live cells in the NanoBRET® assay.

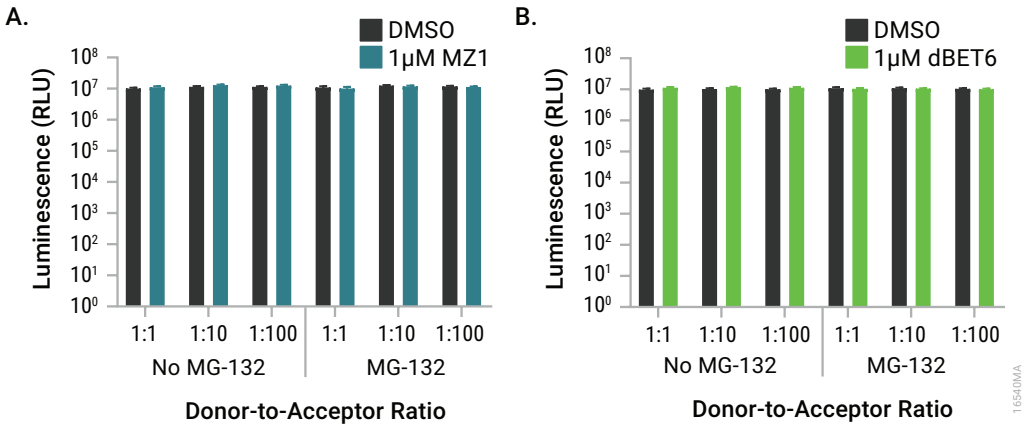

Figure 6. Example data for multiplexing the NanoBRET™ Ternary Complex Assays with CellTiter-Glo® 2.0 Cell Viability Assay. Panel A. Experimental samples for the NanoBRET™ VHL Ternary Complex Assay from Figure 3 were multiplexed with the CellTiter-Glo® 2.0 Assay to measure cell viability. There was no effect on cell viability observed with either MG-132 or MZ1 treatment. **Panel B.** Experimental samples for the NanoBRET™ CRBN Ternary Complex Assay from Figure 4 were multiplexed with the CellTiter-Glo® 2.0 Assay to measure cell viability. There was no effect on cell viability observed with either MG-132 or dBET6 treatment.

8.B. Composition of Buffers and Solutions

Cell Culture Medium

90%	DMEM (GIBCO Cat.#11995)
10%	fetal bovine serum (Seradigm Cat.# 89510-194)

Assay Medium

96%	Opti-MEM™ I Reduced Serum Medium, no phenol red (Thermo Fisher Cat.# 11058021)
4%	fetal bovine serum (Seradigm Cat.# 89510-194)

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8.C. References

1. Riching, K.M. *et al.* (2018) Quantitative live-cell kinetic degradation and mechanistic profiling of PROTAC mode of action. *ACS Chem. Biol.* **13**, 2758–70.
2. Machleidt, T. *et al.* (2015) NanoBRET—A novel BRET platform for the analysis of protein-protein interactions. *ACS Chem. Biol.* **10**, 1797–1804.
3. Zengerle, M., Chan, K.H. and Ciulli, A. (2015) Selective small molecule induced degradation of the BET bromodomain protein BRD4. *ACS Chem. Biol.* **10**, 1770–7.
4. Winter, G.E. *et al.* (2017) BET bromodomain proteins function as master transcription elongation factors independent of CDK9 recruitment. *Mol. Cell* **67**, 5–18.

8.D. Related Products

Detection Systems and Reagents

Product	Size	Cat.#
NanoBRET™ Nano-Glo® Detection System*	200 assays	N1661
NanoBRET™ Nano-Glo® Kinetic Detection System*	200 assays	N2583
NanoBRET™ Nano-Glo® Substrate*	50µl	N1571
HaloTag® NanoBRET™ 618 Ligand	20µl	G9801

*Additional kit sizes are available.

Vectors

Product	Size	Cat.#
HaloTag®-VHL Fusion Vector	20µg	N2731
HaloTag®-CRBN Fusion Vector	20µg	N2691
pFN31A *Nluc* CMV-Hygro Flexi® Vector	20µg	N1311
pFN31K *Nluc* CMV-neo Flexi® Vector	20µg	N1321
pFC32A *Nluc* CMV-Hygro Flexi® Vector	20µg	N1331
pFC32K *Nluc* CMV-neo Flexi® Vector	20µg	N1341
pNLF1-N [CMV/Hygro] Vector	20µg	N1351
pNLF1-C [CMV/Hygro] Vector	20µg	N1361
NanoLuc®-BRD4 FL Fusion Vector	20µg	N1691
NanoBRET™ Positive Control	2 × 20µg	N1581
HaloTag® Control Vector	20µg	G6591

Transfection Reagents

Product	Size	Cat.#
FuGENE® HD Transfection Reagent	1ml	E2311
	5 × 1ml	E2312

NanoBRET™ PPI Kits and Reagents

Product	Size	Cat.#
NanoBRET™ Proteasomal Recruitment Starter Kit	1 each	ND2730
NanoBRET™ Ubiquitination Starter Kit	1 each	ND2690
NanoBRET™ PPI Control Pair (p53, MDM2)	1 each	N1641

Cell Viability Assay

Product	Size	Cat.#
CellTiter-Glo® 2.0 Cell Viability Assay	10ml	G9241
	100ml	G9242
	500ml	G9243

Multimode Detection Instrument

Product	Size	Cat.#
GloMax® Discover System	1 each	GM3000

Intracellular LgBiT Expression Reagents

Product	Size	Cat.#
LgBiT Expression Vector	20µg	N2681
HEK293 LgBiT Cell Line	2 × 1ml	N2672

Cell Lines Available upon Request

Product	Cat.#
HEK293 HaloTag-VHL Stable Cell Line	Please enquire
HEK293 HaloTag-CRBN Stable Cell Line	Please enquire

Please enquire at: **www.promega.com/c/global/forms/contact-tailored-rd-solutions-form/**

9. Summary of Changes

The following changes were made to the 1/24 revision of this document:

1. Updated catalog numbers in Section 5.A.
2. Made minor text edits.

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[d]U.S. Pat. Nos. 8,557,970, 8,669,103, 9,777,311, 9,840,730, 9,951,373, 10,233,485, 10,633,690, 10,844,422, 11,365,436; European Pat. Nos. 2456864, 2635595, 2990478, 3181687, 3409764; Japanese Pat. Nos. 6038649, 6155424, 6227615, 6374420, 6539689; and other patents and patents pending.

[e]U.S. Pat. No. 8,809,529, European Pat. No. 2635582, Japanese Pat. No. 5889910 and other patents and patents pending.

[f]U.S. Pat. Nos. 7,425,436, 7,935,803, 8,466,269, 8,742,086, 8,420,367, 8,748,148, 9,416,353, 9,593,316 and other patents and patents pending.

[g]U.S. Pat. Nos. 10,067,149 and 10,024,862, European Pat. No. 2932267, Japanese Pat. Nos. 6751294 and 7092691 and other patents and patents pending.

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