TECHNICAL MANUAL

Intracellular TE Nano-Glo® Substrate/Inhibitor and Intracellular TE Nano-Glo® Vivazine™/Inhibitor

Instructions for use of Products
N2160, N2161, N2162, N2200, N2201

2/20
TM610

Intracellular TE Nano-Glo® Substrate/Inhibitor and Intracellular TE Nano-Glo® Vivazine™/Inhibitor

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

The Intracellular TE Nano-Glo® Substrate/Inhibitor[a–e] and Intracellular TE Nano-Glo® Vivazine™/Inhibitor[a–e] are designed for use with NanoBRET™ Target Engagement (TE) Assays. These target engagement assays are based on the NanoBRET™ System, an energy transfer technique designed to measure molecular proximity in living cells, and to measure compound binding at select target proteins within intact cells (1). Measurement of NanoBRET™ signals for target-NanoLuc® luciferase fusion proteins located inside living cells requires three key components: a NanoBRET™ tracer, a NanoLuc® substrate and an extracellular NanoLuc® inhibitor. NanoLuc® substrates are designed to produce adequate luminescence for BRET measurements over a specified range of time (i.e., short or long term). The extracellular NanoLuc® inhibitor is an impermeable molecule designed to quench any NanoLuc® signal derived from the extracellular environment. The combination of these three key components allows robust measurement of BRET signals originating from NanoLuc® target fusions that are located exclusively within live, intact cells.

There are multiple furimazine-based substrates available for NanoLuc® luciferase, depending on the desired duration of BRET measurement (Figure 1). For experiments lasting less than two hours, NanoBRET™ Nano-Glo® Substrate provides a bright luminescent signal (Figure 1, Panel A). The NanoBRET™ Nano-Glo® Substrate is optimal for all end-point measurements of live-cell target engagement at equilibrium, and can also be used for kinetic experiments where NanoBRET™ is being measured for short durations in real time.

However, the duration of live-cell measurements using NanoBRET™ Nano-Glo® Substrate is limited due to degradation of the substrate and subsequent signal decay that reduces signal quality and sensitivity. During live-cell experiments, NanoBRET™ Nano-Glo® Substrate undergoes both enzymatic and nonenzymatic turnover, and the presence of serum enhances the rate of signal decay.

To address this issue and enable NanoBRET™ measurements over more than two hours, an extended lifetime substrate such as Nano-Glo® Vivazine™ Substrate may be used in place of the conventional NanoBRET™ Nano-Glo® Substrate (Figure 1, Panel B). Nano-Glo® Vivazine™ Substrate is a derivative of NanoBRET™ Nano-Glo® Substrate that produces luminescence over many hours. Hydrolysis of Vivazine™ Substrate by cellular esterases leads to a steady release of the substrate for NanoLuc® luciferase. In addition, the Nano-Glo® Vivazine™ Substrate is prepared with a proprietary formulation that reduces autoluminescence. Depending on the duration of measurement, both conventional NanoBRET™ Nano-Glo® Substrate and Nano-Glo® Vivazine™ Substrate provide strong options to measure NanoBRET™ signals originating from live, intact cells, specifically when paired with the Extracellular NanoLuc® Inhibitor.

This technical manual covers the NanoBRET™ detection step of the NanoBRET™ TE Assay for the use of short- or long-duration NanoLuc® substrates. The NanoBRET™ Target Engagement Assay (Figure 2, Panel A) measures the apparent affinity of test compounds by competitive displacement of a NanoBRET™ Tracer reversibly bound to a NanoLuc® luciferase fusion protein in cells (2,3). In the first step of the NanoBRET™ TE Assay (Figure 2, Panel B), a fixed concentration of tracer is added to cells expressing the desired NanoLuc® fusion protein to generate a BRET reporter complex. Introduction of competing compounds results in a dose-dependent decrease in NanoBRET™ energy transfer, which allows quantitation of the apparent intracellular affinity of the target protein for the test compound (Figure 2, Panel C). The NanoBRET™ TE Assay has been applied successfully to study multiple target classes inside living cells including histone deacetylases (2), the BET family of the bromodomains (2), kinases (2,3), histone lysine demethylases (4), and E3 ligases (5).

For complete details regarding the setup of NanoBRET™ TE Assays and assay design considerations, consult: *NanoBRET™ Target Engagement Intracellular Kinase Assay, Adherent Format Technical Manual*, #TM598; *NanoBRET™ Target Engagement Intracellular Kinase Assay, Nonbinding Surface Format Technical Manual*, #TM603; or Robers *et al.* (6). For additional details regarding the use of extended duration live-cell NanoLuc® Substrates, consult the *Nano-Glo® Endurazine™ and Vivazine™ Live Cell Substrates Technical Manual*, #TM550.

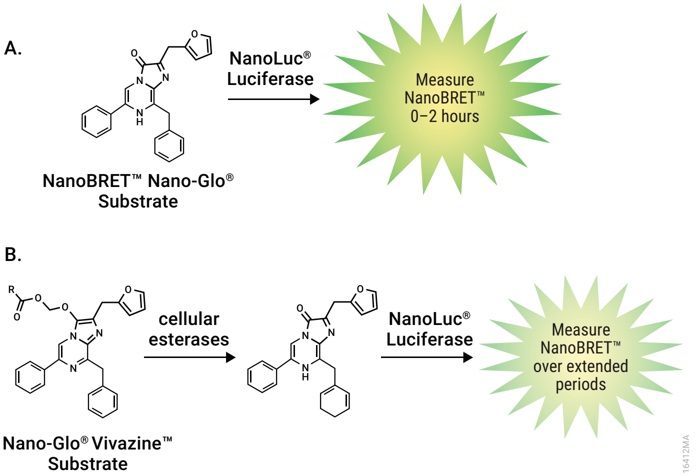

Figure 1. Overview of the NanoLuc® substrates available for the NanoBRET™ Target Engagement Assay. Panel A. Conventional NanoBRET™ Nano-Glo® Substrate quickly generates bright luminescence that can be used to measure a NanoBRET™ signal over short durations (0–2 hours). **Panel B.** Nano-Glo® Vivazine™ Substrate is hydrolyzed by cellular esterases and releases active substrate steadily throughout the experiment. Nano-Glo® Vivazine™ Substrate generates stable luminescence than can be used to measure a NanoBRET™ signal over extended periods (up to 24 hours) depending on target expression level.

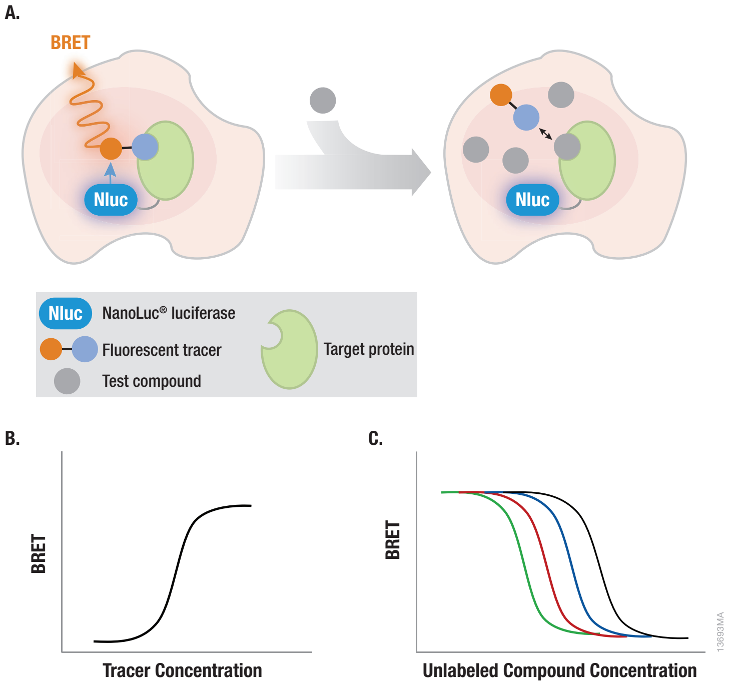

Figure 2. Illustration of the NanoBRET™ TE Assay. Panel A. Compound engagement is measured in a competitive format using a cell-permeable fluorescent NanoBRET™ tracer. Binding of the test compound results in a loss of NanoBRET™ signal between the target protein and the tracer inside intact cells. **Panel B.** The affinity of the NanoBRET™ tracer is determined for each target protein. For analysis of target engagement by a test compound, cells are treated with a fixed concentration of NanoBRET™ tracer that is near the EC_{50} value of the NanoBRET™ tracer dose response curve. **Panel C.** To determine test compound affinity, cells are titrated with varying concentrations of the test compound in the presence of a fixed concentration (EC_{50}–EC_{80}) of tracer.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Intracellular TE Nano-Glo® Substrate/Inhibitor	**100 assays**	**N2162**

Provides sufficient reagent for 100 assays. Includes:

- 50µl NanoBRET™ Nano-Glo® Substrate
- 17µl Extracellular NanoLuc® Inhibitor

PRODUCT	SIZE	CAT.#
Intracellular TE Nano-Glo® Substrate/Inhibitor	**1,000 assays**	**N2160**

Provides sufficient reagent for 1,000 assays. Includes:

- 330µl NanoBRET™ Nano-Glo® Substrate
- 110µl Extracellular NanoLuc® Inhibitor

PRODUCT	SIZE	CAT.#
Intracellular TE Nano-Glo® Substrate/Inhibitor	**10,000 assays**	**N2161**

Provides sufficient reagents for 10,000 assays. Includes:

- 3.3ml NanoBRET™ Nano-Glo® Substrate
- 1.1ml Extracellular NanoLuc® Inhibitor

PRODUCT	SIZE	CAT.#
Intracellular TE Nano-Glo® Vivazine™/Inhibitor	**1,000 assays**	**N2200**

Provides sufficient reagents for 1,000 assays. Includes:

- 1.65ml Nano-Glo® Vivazine™ Substrate
- 110µl Extracellular NanoLuc® Inhibitor

PRODUCT	SIZE	CAT.#
Intracellular TE Nano-Glo® Vivazine™/Inhibitor	**10,000 assays**	**N2201**

Provides sufficient reagents for 10,000 assays. Includes:

- 16.5ml Nano-Glo® Vivazine™ Substrate
- 1.1ml Extracellular NanoLuc® Inhibitor

Storage Conditions: Store NanoBRET™ Nano-Glo® Substrate and Nano-Glo® Vivazine™ Substrate at –30°C to –10°C. The NanoBRET™ Nano-Glo® Substrate and Nano-Glo® Vivazine™ Substrate will not freeze at –20°C. Minimize the amount of time that each stock is kept at room temperature and exposed to light.

3. Instrument Requirements and Setup

To perform NanoBRET™ TE Assays, a luminometer capable of sequentially measuring dual-wavelength windows is required. This is accomplished using filters; we recommend using a band pass (BP) filter for the donor signal and a long pass filter (LP) for the acceptor signal to maximize sensitivity.

- The NanoBRET™ bioluminescent donor emission occurs at 460nm. To measure this 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 410nm to 490nm range.
  Note: A BP filter is preferred for the donor signal measurement to selectively capture the signal peak and avoid measuring any acceptor peak bleedthrough. However, a short pass (SP) filter that covers the 460nm area also can be used. This may 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 peak emission occurs at approximately 590–610nm. To measure the acceptor signal, we recommend a long pass filter starting at 600–610nm.

For instruments using mirrors, select the luminescence mirror. An integration time of 0.2–1 second is typically sufficient. Ensure that the gain on the PMT is optimized to capture the highest donor signal without reaching instrument saturation.

Instruments capable of dual-luminescence measurements are either equipped with a filter selection or the filters can be purchased and added separately. Consult with your instrument manufacturer to determine if the proper filters are installed or for the steps 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:

1. 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.
2. BMG Labtech CLARIOstar® with preloaded filters for donor 450nm/80nm BP and acceptor 610nm LP
3. Thermo Varioskan® with filters obtained from Edmunds Optics, using donor 450nm CWL, 25mm diameter, 80nm FWHM, Interference Filter and acceptor 1 inch diameter, RG-610 Long Pass Filter.

Another instrument capable of measuring dual luminescence is the PerkinElmer Envision® Multilabel Reader with the following recommended setup:

- 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

4. Live-Cell NanoBRET™ Target Engagement Assay Protocol

This general protocol can be used to measure NanoBRET™ for cells expressing a NanoLuc® fusion to a target of interest. Those cells can be stably expressing the NanoLuc® fusion or may be expressing the fusion transiently after transfection. For more details on preparation of cells or example set up of a NanoBRET™ Target Engagement Assay, consult technical manuals #TM598 or #TM603, or Robers *et al*. (6).

Materials to be Supplied by the User

- Opti-MEM® I Reduced Serum Medium, without phenol red (Life Technologies Cat.# 11058-021)
- cells (~100µl) expressing a NanoLuc® fusion to a target of interest, pre-equilibrated with a NanoBRET™ tracer, and seeded into either a:
  a. white, tissue-culture treated, 96-well (Corning® Cat.# 3917) or 384-well (Corning® Cat.# 3570) plate, **or** a
  b. white, nonbinding surface, 96-well (Corning® Cat.# 3600) or 384-well (Corning® Cat.# 3574) plate.

  Note: For optimal performance, the cells should be seeded in a low serum medium without phenol red, such as Opti-MEM® I reduced serum medium. Serum concentrations up to 1% in the cell culture medium are well tolerated without significantly increasing substrate autoluminescence.
- detection instrument capable of measuring NanoBRET™ wavelengths (e.g., GloMax® Discover System, Cat.# GM3000; see Section 3.)

The volumes specified for the NanoBRET™ Target Engagement protocol are for 96-well plates. Table 1 lists the assay volumes used for both 96- and 384-well plates. Modify the reagent volumes in Section 4.A as listed in Table 1 if 384-well plates are used.

Table 1. Volumes of NanoBRET™ Assay Components Used for Multiwell Plates.

	Volume Per Well	
Assay Component	**96-Well Plate**	**384-Well Plate**
Opti-MEM® reduced serum medium without phenol red, with NanoLuc®-expressing cells and NanoBRET™ tracer	100µl	40µl
3X Complete Substrate plus Inhibitor Solution (Section 4.A)	50µl	20µl

4.A. Measuring NanoBRET™ Using a 3X Stock Solution

1. Remove plate with cells expressing the NanoLuc® fusion of interest from the incubator and equilibrate to room temperature for 15 minutes. Alternatively, if the cells are already at room temperature, proceed directly to Step 2.
2. Prepare 3X Complete Substrate plus Inhibitor Solution just before measuring BRET.
    a. For short-duration measurements (≤2 hours), prepare a solution consisting of a 1:166 dilution of NanoBRET™ Nano-Glo® Substrate plus a 1:500 dilution of Extracellular NanoLuc® Inhibitor in Opti-MEM® reduced serum medium, without phenol red. See Table 2 for examples.
    b. For long-duration measurements (>2 hours and up to 24 hours), prepare a solution consisting of a 1:33 dilution of Nano-Glo® Vivazine Substrate plus a 1:500 dilution of Extracellular NanoLuc® Inhibitor in Opti-MEM® reduced serum medium, without phenol red. See Table 3 for examples.

Table 2. Volumes of Complete Substrate Solution Components Used for Short-Duration Experiments.

Assay Component	96-Well Plate	384-Well Plate
NanoBRET™ Nano-Glo® Substrate	30µl	48µl
Extracellular NanoLuc® Inhibitor	10µl	16µl
Opti-MEM® Reduced Serum Medium (no phenol red)	4,960µl	7,936µl
Total Volume	**5,000µl**	**8,000µl**

Table 3. Volumes of Complete Substrate Solution Components Used for Long-Duration Experiments.

Assay Component	**96-Well Plate**	**384-Well Plate**
Nano-Glo® Vivazine Substrate	150µl	240µl
Extracellular NanoLuc® Inhibitor	10µl	16µl
Opti-MEM® reduced serum medium without phenol red	4,840µl	7,744µl
Total Volume	**5,000µl**	**8,000µl**

Note: Use either of these 3X Complete Substrate plus Inhibitor Solutions within 1.5 hours. Discard any unused solution.

3. Add 50µl of 3X Complete Substrate plus Inhibitor Solution to each well of the 96-well plate. Incubate for 2–3 minutes at room temperature.
4. Measure donor emission wavelength (e.g., 450nm) and acceptor emission wavelength (e.g., 610nm or 630nm) using the GloMax® Discover System or other NanoBRET™ Assay-compatible luminometer (see Section 3.B).

Notes:

If using **NanoBRET™ Nano-Glo® Substrate**, we recommend measuring BRET within 10 minutes after adding NanoBRET™ Nano-Glo® Substrate plus Extracellular NanoLuc® Inhibitor Solution. You can measure BRET kinetically for up to 2 hours, but there will be some loss of luminescent signal over time.

If using **Nano-Glo® Vivazine™ Substrate**, we recommend measuring BRET within 5 hours after adding Nano-Glo® Vivazine™ Substrate plus Extracellular NanoLuc® Inhibitor Solution. You can measure BRET kinetically for extended periods up to 24 hours depending on the amount of NanoLuc® protein in the assay well, but there will be some loss of luminescent signal over time.

4.B. Determining BRET Ratio

1. To generate raw BRET ratio values, divide the acceptor emission value (e.g., 610nm) by the donor emission value (e.g., 450nm) for each sample.

   Optional: To correct for background, subtract the BRET ratio in the absence of tracer (average of no-tracer control samples) from the BRET ratio of each sample.

2. **NanoBRET™ ratio equation:** Convert raw BRET units to milliBRET units (mBU) by multiplying each raw BRET value by 1,000.

$$\text{BRET ratio} = \frac{\text{Acceptor}_{\text{Sample}}}{\text{Donor}_{\text{Sample}}} \times 1{,}000$$

NanoBRET™ ratio equation, including optional background correction:

$$\text{BRET ratio} = \left[\frac{\text{Acceptor}_{\text{Sample}}}{\text{Donor}_{\text{Sample}}} - \frac{\text{Acceptor}_{\text{No Tracer Control}}}{\text{Donor}_{\text{No Tracer Control}}} \right] \times 1{,}000$$

4.C. Comparing Performance of NanoLuc® Substrates

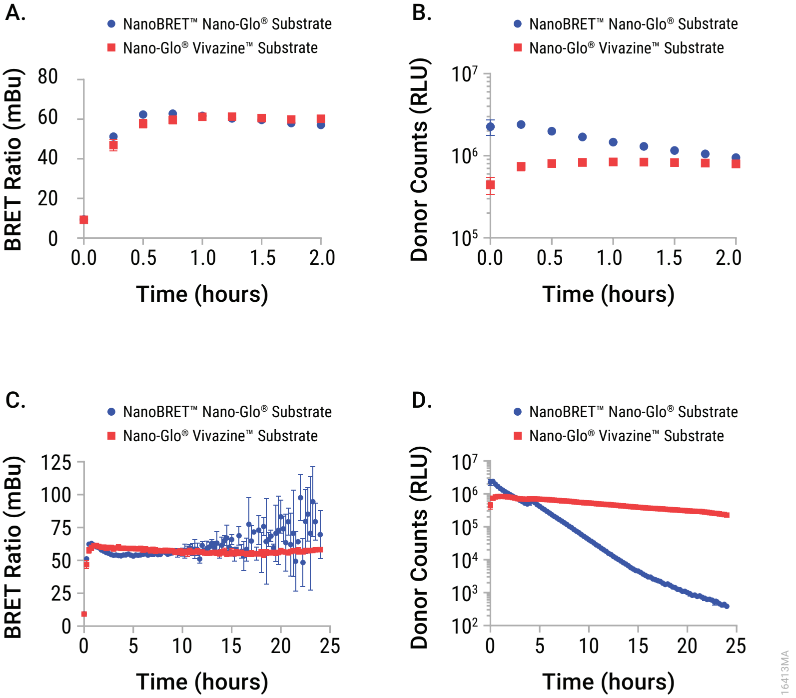

Figure 3. Performance of NanoBRET™ Nano-Glo® Substrate and Nano-Glo® Vivazine™ Substrate in a NanoBRET™ Target Engagement Assay. Adherent HEK293 cells expressing the DDR1-NanoLuc® fusion protein were mixed in a 96-well plate with NanoBRET™ Tracer K-4 (0.13µM). Equilibration of the tracer and establishment of the BRET signal was measured over time by adding 3X Complete Substrate plus Inhibitor Solution containing either conventional NanoBRET™ Nano-Glo® Substrate or Nano-Glo® Vivazine™ Substrate. BRET measurements were measured kinetically at 15-minute intervals using a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Donor counts were plotted as relative luminescence units (RLU) and BRET values were plotted as milliBRET units (mBU). Over the first 2 hours of the time course, BRET (**Panel A**) is equivalent regardless of the substrate, while donor signal (**Panel B**) is significantly stronger for conventional NanoBRET™ Nano-Glo® Substrate compared to Nano-Glo® Vivazine™ Substrate. After 2 hours, Nano-Glo® Vivazine™ Substrate demonstrates significantly lower BRET signal variability at later timepoints compared to conventional NanoBRET™ Nano-Glo® Substrate (**Panel C**), as well as a slower donor signal decay rate (**Panel D**). Assays were equilibrated for 2 hours (**Panels A and B**) or 24 hours (**Panels C and D**).

5. 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	**Possible Causes and Comments**
Nano-Glo® Vivazine™ RLUs significantly reduced compared to RLUs obtained with conventional NanoBRET™ Nano-Glo® Substrate	Nano-Glo® Vivazine™ Substrate enables BRET reads over several hours by providing a steady release of active substrate (furimazine) throughout the experiment. As a consequence, the concentration of active substrate at a given time point is substantially lower than the concentration provided by conventional NanoBRET™ Nano-Glo® Substrate. If signals are too dim, increase the strength of transfection or switch to a stronger promoter if possible.
Noisy data	Poor expression levels of the NanoLuc® fusion protein. Not all fusion proteins express identically and expression may vary in different cell types. If poor expression is suspected, determine the luminescence (RLU) from the donor (450nm) and acceptor (610nm) channels for cells expressing the NanoLuc® fusion (the signal) and compare that to the donor and acceptor RLU in the absence of cells expressing NanoLuc® (the background). If the signal-to-background ratio is less than 100, consider optimizing transfection conditions. Transfection optimization could involve increasing the ratio of the NanoLuc® fusion vector to the transfection carrier DNA while keeping the total DNA in the transfection mixture fixed. Alternatively, consider switching to a strong promoter, if possible. Lastly, for best performance using transient transfection, choose cells that have been freshly passaged (ideally within 24–48 hours) and are at ~80–95% confluency.

6. Appendix

6.A. Factors that Influence Signal Intensity and Signal Decay

Signal intensity will be determined by the amount of substrate and the amount of active NanoLuc® luciferase present at a given time point. When using Nano-Glo® Vivazine™ Extended Lifetime Substrate, active substrate is steadily released throughout a time-course experiment, a process catalyzed by cellular esterases. Immediately after adding Nano-Glo® Vivazine™ Substrate to cells, the active substrate will begin to accumulate, increasing enzyme-catalyzed luminescence. After the initial accumulation, substrate levels will reach a steady state, where production is balanced by degradation. Both enzymatic and nonenzymatic pathways mediate turnover of active substrate. NanoLuc® luciferase acts on the substrate, with serum components like albumin contributing to a much lower extent. The substrate also degrades via nonenzymatic pathways in aqueous solutions. After achieving a steady state, the substrate levels will decrease when the rate of turnover exceeds the rate of production, leading to signal decay for a constant amount of active luciferase.

The amount of active NanoLuc® luciferase will depend on several factors. For stable cell lines, expression will depend upon the promoter regulating expression of the NanoLuc® fusion, the rate of target degradation and the cell-seeding density. For transient transfection, expression will depend upon the amount of NanoLuc® fusion DNA transfected into the cells, the time over which expression is allowed to occur, the promoter controlling expression of the NanoLuc® fusion, the rate of target degradation and the cell-seeding density. Though all of these factors will determine the luminescence signal over time, the rate of signal decay should be similar for a given substrate and over a similar range of NanoLuc® expression.

6.B. Reconfiguring the Complete Substrate plus Inhibitor Solution as a 2X Reagent

Over extended periods of time (up to 24 hours), evaporation from assay wells can be significant. To lessen the impact of evaporation, the Complete Substrate plus Inhibitor Solution used to measure NanoLuc® luciferase can be used as a 2X solution instead of a 3X solution, thus increasing the total assay volume at the detection step. Tables 4, 5 and 6 provide assay and 2X reagent preparation examples.

Table 4. Volumes of NanoBRET™ Assay Components Used with 2X Complete Substrate Plus Inhibitor Solution.

	Volume Per Well	
Assay Component	**96-Well Plate**	**384-Well Plate**
Opti-MEM® reduced serum medium, without phenol red with NanoLuc®-expressing cells and NanoBRET™ tracer	100µl	40µl
2X Complete Substrate plus Inhibitor Solution (see Table 5 or Table 6)	100µl	40µl

6.B. Reconfiguring the Complete Substrate plus Inhibitor Solution as a 2X Reagent (continued)

Table 5. Volumes of Complete 2X Substrate Solution Components for Short-Duration (≤2 hours) Experiments.

Assay Components	96-Well Plate	384-Well Plate
NanoBRET™ Nano-Glo® Substrate	40µl	64µl
Extracellular NanoLuc® Inhibitor	13.3µl	21.3µl
Opti-MEM® reduced serum medium, without phenol red	9,946.7µl	15,914.7µl
Total Volume	**10,000µl**	**16,000µl**

Table 6. Volumes of Complete 2X Substrate Solution Components for Long-Duration (>2 hours) Experiments.

Assay Components	96-Well Plate	384-Well Plate
Nano-Glo® Vivazine™ Substrate	200µl	320µl
Extracellular NanoLuc® Inhibitor	13.3µl	21.3µl
Opti-MEM® reduced serum medium, without phenol red	9,786.7µl	15,658.7µl
Total Volume	**10,000µl**	**16,000µl**

6.C. References

1. Machleidt, T. *et al.* (2015) NanoBRET-A novel BRET platform for the analysis of protein-protein interactions. *ACS Chem. Bio.* **10**, 1797–1804.
2. Robers, M.B. *et al.* (2015) Target engagement and drug residence time can be observed in living cells with BRET. *Nat. Comm.* **6**, 10091.
3. Vasta, J.D. *et al.* (2018) Quantitative, wide-spectrum kinase profiling in live cells for assessing the effect of cellular ATP on target engagement. *Cell Chem. Biol.* **25**, 206–14.
4. Vasquez-Rodriguez, S. *et al.* (2019) Design, synthesis, and characterization of covalent KDM5 inhibitors. *Angew Chem. Int. Ed. Engl.* **58**, 515–19.
5. 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.
6. Robers, M.B. *et al.* (2019) Quantitative, Real-time measurements of intracellular target engagement using energy transfer. *Syst. Chem. Biol.* pp 45–71.

6.D. Related Products

NanoBRET™ Kinase Target Engagement Assays

Product	Size	Cat.#
NanoBRET™ TE Intracellular Kinase Assay K-3*	100 assays	N2600
NanoBRET™ TE Intracellular Kinase Assay K-4*	100 assays	N2520
NanoBRET™ TE Intracellular Kinase Assay K-5*	100 assays	N2500
NanoBRET™ TE Intracellular Kinase Assay K-8*	100 assays	N2620
NanoBRET™ TE Intracellular Kinase Assay K-9*	100 assays	N2630
NanoBRET™ TE Intracellular Kinase Assay K-10*	100 assays	N2640
NanoBRET™ TE Intracellular Kinase Assay K-11*	100 assays	N2650

*Additional assay sizes are available.

NanoBRET™ Kinase Target Engagement Assay Reagents

Product	Size	Cat.#
Tracer Dilution Buffer	50ml	N2191
Transfection Carrier DNA	5 × 20µg	E4881
Transfection Carrier DNA	2 × 100µg	E4882

NanoBRET™ Target Engagement Intracellular HDAC and BET BRD Assays

Product	Size	Cat. #
NanoBRET™ Target Engagement Intracellular HDAC Assays	100 assays	N2080
	1,000 assays	N2081
	10,000 assays	N2090
NanoBRET™ Target Engagement Intracellular BET BRD Assays	100 assays	N2130
	1,000 assays	N2131
	10,000 assays	N2140

Transfection Reagents

Products	Size	Cat. #
FuGENE® HD Transfection Reagent	1ml	E2311
	5 × 1ml	E2312
ViaFect™ Transfection Reagent	0.75ml	E4981
	2 × 0.75ml	E4982

Luminometers

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

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(a) use NanoBRET™-branded energy acceptors (e.g., NanoBRET™ tracers, NanoBRET™ dyes, BRET-optimized HaloTag® ligands) for all determinations of energy transfer activity;

(b) contact Promega to obtain a license for use of the product for energy transfer assays using energy acceptors not manufactured by Promega.

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[d] U.S. Pat. Nos. 10,067,149 and 10,024,862 and other patents and patents pending.

[e] Patent Pending.

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