TECHNICAL MANUAL

NanoBRET™ Target Engagement Intracellular Kinase Assay, K-5

Instructions for Use of Products
N2500, N2501 and N2530

Revised 11/17
TM520

NanoBRET™ Target Engagement Intracellular Kinase Assay, K-5

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 NanoBRET™ Target Engagement (TE) Assay measures compound binding at select target proteins within intact cells. This target engagement assay is based on the NanoBRET™ System, an energy transfer technique designed to measure molecular proximity in living cells (1). The NanoBRET™ Target Engagement Intracellular Kinase Assay[a–g] 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). In the first step of the NanoBRET™ TE Assay, 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.

The NanoBRET™ TE Assay has been applied successfully to study multiple target classes including histone deacetylases and the BET family of the bromodomains. Here, we describe the NanoBRET™ TE Intracellular Kinase Assay, which measures compound binding to a kinase protein fused to NanoLuc® luciferase. As the largest group of enzymes in the human proteome, the kinases are essential to myriad cellular processes from regulation of cell physiology to signal transduction. The NanoBRET™ TE Assay allows for the measurement of compound binding in the presence of cellular factors that are known to impact target engagement potency. The NanoBRET™ TE Intracellular Kinase Assay, K-5, allows the quantitation of apparent intracellular affinity for a test compound to a diverse set of full-length protein kinases expressed inside living cells.

The NanoBRET™ TE Assay uses four key components: an expressed cellular target protein that is fused to the bright NanoLuc® luciferase; a cell-permeable fluorescent NanoBRET™ tracer that specifically binds to the target protein; a substrate for NanoLuc® luciferase; and an extracellular inhibitor for NanoLuc® luciferase. Bioluminescence resonance energy transfer (BRET) is achieved through a nonradiative transfer of the luminescent energy from NanoLuc® luciferase to the fluorescent tracer that is bound to the target protein-NanoLuc® fusion (Figure 1, Panels A and B). Compounds that are applied to the cells and specifically engage the intracellular target protein-NanoLuc® fusion will result in a decrease in BRET (Figure 1, Panels A and C). To ensure accurate assessment of intracellular target engagement, an extracellular NanoLuc® inhibitor is used to mitigate any NanoLuc® signal that may arise from cells compromised during handling, while not adversely affecting NanoLuc® luciferase expressed within healthy living cells. An overview of the NanoBRET™ Target Engagement Assay workflow is shown in Figure 2.

The NanoBRET™ TE Assays have been optimized to use a blue-shifted NanoLuc® donor and a red-shifted fluorescent tracer acceptor (NanoBRET™ 590) that have minimal spectral overlap within the assay (Figure 3). This results in an optimized signal:background ratio and hence an optimized NanoBRET™ ratio.

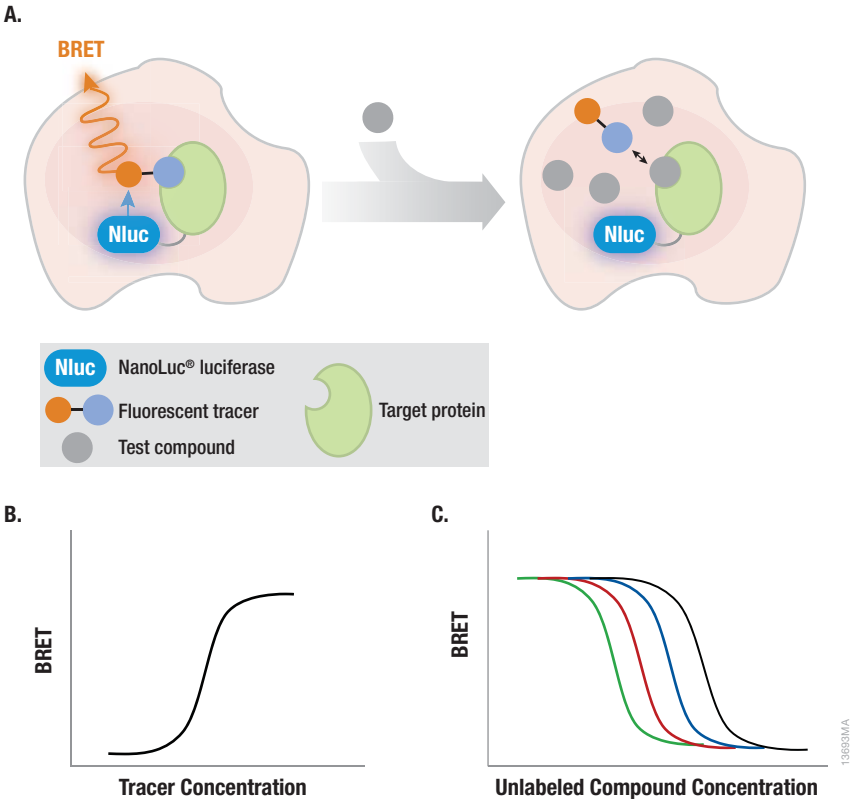


Figure 1. Illustration of the NanoBRET™ TE Assay. Panel A. Compound engagement is measured in a competitive format using a cell-permeable fluorescent NanoBRET™ tracer. Binding of the test compound results in a loss of NanoBRET™ signal between the target protein and the tracer inside intact cells. **Panel 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.

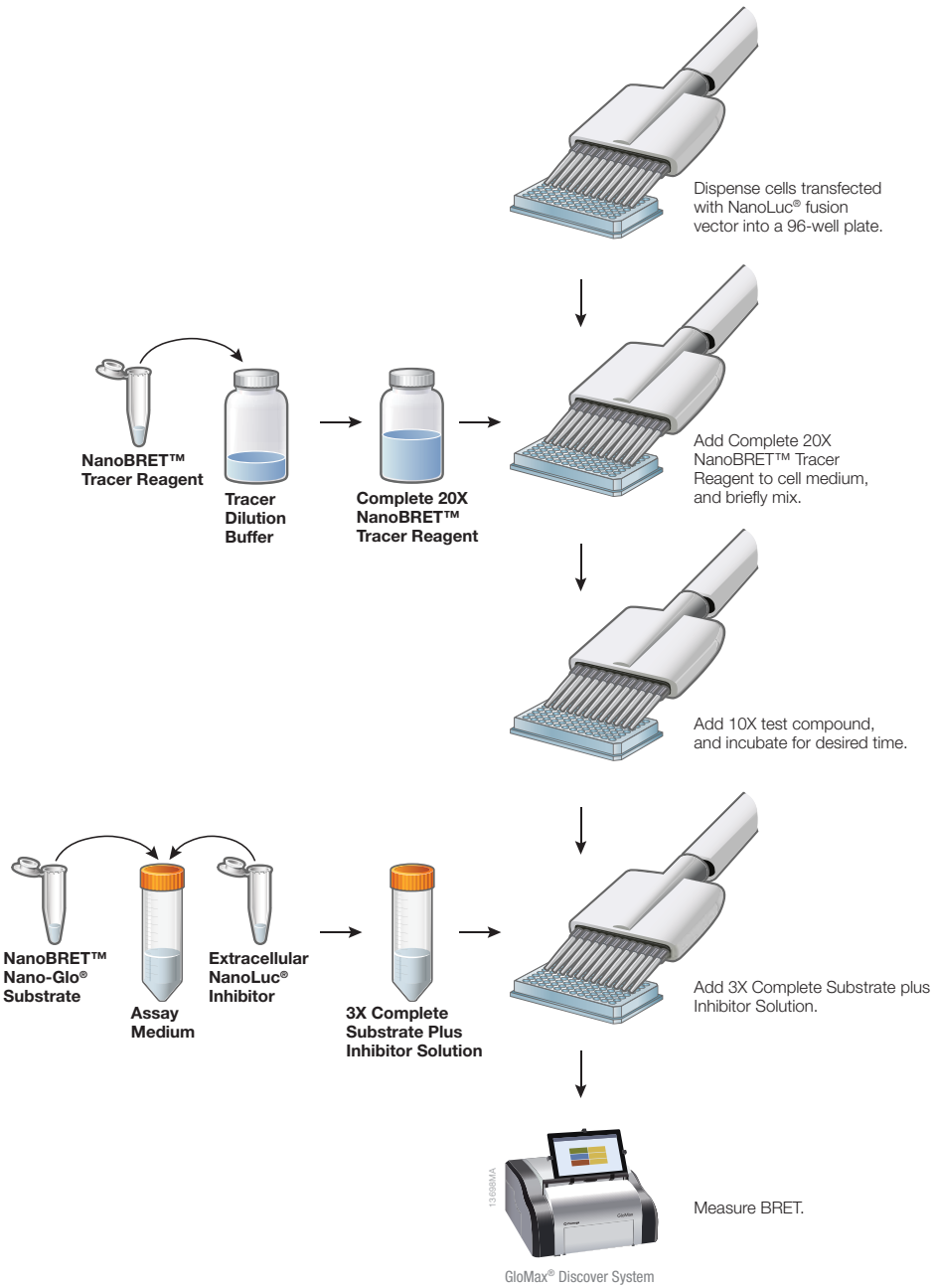


Figure 2. Overview of the NanoBRET™ Target Engagement Intracellular Assay.

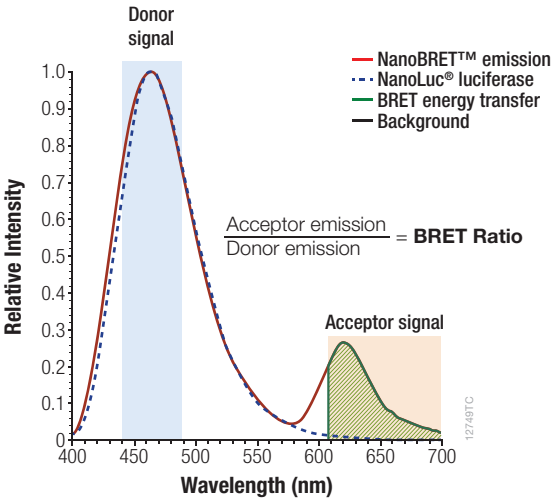


Figure 3. Spectral separation of the NanoLuc® emission (460nm) and fluorescent tracer emission (618nm), and calculation of the NanoBRET™ ratio.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
NanoBRET™ TE Intracellular Kinase Assay, K-5	**100 assays**	**N2500**

This assay system is sufficient for 100 assays performed in 96-well plates. This system can also be used in 384-well plates for a total of 250 assays. It includes:

- 20µg BTK-NanoLuc® Fusion Vector
- 20µg Transfection Carrier DNA
- 55µl NanoBRET™ Tracer K-5, 0.4mM
- 5ml Tracer Dilution Buffer
- 50µl NanoBRET™ Nano-Glo® Substrate
- 17µl Extracellular NanoLuc® Inhibitor (30mM in DMSO)

PRODUCT	SIZE	CAT.#
NanoBRET™ TE Intracellular Kinase Assay, K-5	**1,000 assays**	**N2501**

This assay system is sufficient for 1,000 assays performed in 96-well plates. This system can also be used in 384-well plates for a total of 2,500 assays. It includes:

- 20µg BTK-NanoLuc® Fusion Vector
- 100µg Transfection Carrier DNA
- 550µl NanoBRET™ Tracer K-5, 0.4mM
- 5ml Tracer Dilution Buffer
- 330µl NanoBRET™ Nano-Glo® Substrate
- 110µl Extracellular NanoLuc® Inhibitor (30mM in DMSO)

2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
NanoBRET™ TE Intracellular Kinase Detection Reagents, K-5	**10,000 assays**	**N2530**

This assay system is sufficient for 10,000 assays performed in 96-well plates. This system can also be used in 384-well plates for a total of 25,000 assays. It includes:

- 5.5ml NanoBRET™ Tracer K-5, 0.4mM
- 50ml Tracer Dilution Buffer
- 3.3ml NanoBRET™ Nano-Glo® Substrate
- 1.1ml Extracellular NanoLuc® Inhibitor (30mM in DMSO)

Storage Conditions: Store the entire NanoBRET™ TE Intracellular Kinase Assay, K-5, and NanoBRET™ TE Intracellular Kinase Detection Reagents, K-5, at less than –65°C. Alternatively, store NanoBRET™ Tracer K-5, 0.4mM, at less than –65°C and all other components at less than –10°C. Avoid multiple freeze-thaw cycles of the vector components. Store NanoBRET™ Tracer K-5, 0.4mM, NanoBRET™ Nano-Glo® Substrate and Extracellular NanoLuc® Inhibitor protected from light.

Available Separately

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

Includes:

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

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

Includes:

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

PRODUCT	SIZE	CAT.#
Tracer Dilution Buffer	**50ml**	**N2191**
Transfection Carrier DNA	**100µg**	**E4881**

3. Before You Begin

3.A. Preparing NanoBRET™ Expression Vectors

The amount of each plasmid DNA provided with the system is sufficient for a limited number of experiments. We strongly recommend that each plasmid is further propagated as transfection-ready (i.e., low-endotoxin) DNA. Due to the apparent toxicity of some kinase gene sequences, we recommend the use of *E. coli* strain JM109 for propagation of kinase-NanoLuc® fusions. Follow standard protocols for plasmid transformation into *E. coli* for archival storage, vector propagation and tissue-culture-grade DNA preparation. For each vector, the fusion protein is constitutively expressed by a CMV promoter and includes a kanamycin expression cassette to select for the plasmid during bacterial propagation. For vector sequence information, visit: **https://www.promega.com/products/cell-signaling/kinase-target-engagement/nanobret-te-intracellular-kinase-assay/**

3.B. 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.

1. 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 bleedthrough into the acceptor peak, which could compress the ratio calculation, reducing the assay window.

2. 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.

Instruments capable of dual-luminescence measurements are either 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 on the 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 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.

3.B. Instrument Requirements and Setup (continued)

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. NanoBRET™ TE Intracellular Kinase Assay, K-5 Protocol

Materials to be Supplied by the User

(Media Compositions are supplied in Section 7.E.)

- HEK293 or similar cultured mammalian cells
- Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Cat.# 11995-065)
- fetal bovine serum (HyClone Cat.# SH30070.03, Seradigm Cat.# 1500-050)
- Opti-MEM® I Reduced Serum Medium, no phenol red (Life Technologies Cat.# 11058-021)
- white, nonbinding surface (NBS) 96-well plates (Corning Cat.# 3600) or 384-well plates (Corning Cat.# 3574)
- tissue culture equipment and reagents
- polypropylene plasticware (**Note: Do not use polystyrene plasticware for this assay.**)
- 0.05% Trypsin/EDTA (Thermo Fisher Cat.# 25300)
- FuGENE® HD Transfection Reagent (Cat.# E2311)
- DMSO (Sigma Cat.# 2650)
- detection instrument capable of measuring NanoBRET™ wavelengths (e.g., GloMax® Discover System [Cat.# GM3000]; see Section 3.B)

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 Sections 4.A–D as listed in Table 1 if 384-well plates are used.

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

	Volume Per Well	
Add Tracer and Test Compound	**384-Well Plate**	**96-Well Plate**
Assay Medium with transfected cells	34µl	85µl
Complete 20X NanoBRET™ Tracer Reagent	2µl	5µl
10X Test Compound	4µl	10µl
Assay volume	**40µl**	**100µl**
Add NanoBRET™ Assay Reagents		
3X Complete Substrate plus Inhibitor Solution (Section 4.D)	20µl	50µl
Final assay volume	**60µl**	**150µl**

4.A. Transient Transfection of HEK293 Cells with BTK-NanoLuc® Fusion Vector DNA

1. Cultivate HEK293 cells (or desired cell type) appropriately prior to assay. **Note:** This protocol has been optimized for HEK293 cells. If other cell types are used, optimize the transfection conditions.
2. Remove medium from cell flask by aspiration, trypsinize and allow cells to dissociate from the flask.
3. Neutralize trypsin using Cell Culture Medium and centrifuge at 200 × *g* for 5 minutes to pellet cells.
4. Aspirate medium and resuspend cells in Cell Culture Medium.
5. Adjust density to 2×10^5 cells/ml using Cell Culture Medium.
6. If HEK293 cells are used, prepare lipid:DNA complexes as follows:
    a. Prepare a 10µg/ml solution of DNA in Assay Medium that consists of the following ratios: 9.0µg/ml of Transfection Carrier DNA, 1.0µg/ml of NanoLuc® fusion DNA and 1ml of Assay Medium. To accurately dilute the NanoLuc® fusion DNA, serially dilute the fusion vector with Transfection Carrier DNA to maintain the same amount of DNA (e.g., 10µg).
    b. Mix thoroughly.
    c. Add 30µl of FuGENE® HD Transfection Reagent into each milliliter of DNA mixture to form lipid:DNA complex. Ensure that the FuGENE® HD Transfection Reagent does not touch the plastic side of the tube; pipet directly into the liquid in the tube.
    d. Mix by inversion 5–10 times.
    e. Incubate at ambient temperature for 20 minutes to allow complexes to form.
7. In a sterile, conical tube, mix 1 part of lipid:DNA complex (e.g., 1ml) with 20 parts of HEK293 cells (e.g., 20ml) in suspension at 2×10^5 cells/ml. Mix gently by inversion 5 times.

    Note: Larger or smaller bulk transfections should be scaled accordingly, using this 20:1 cells to lipid:DNA complex ratio.

4.A. Transient Transfection of HEK293 Cells with BTK-NanoLuc® Fusion Vector DNA (continued)

8. Dispense cells + lipid:DNA complex into a sterile tissue culture flask and incubate 20–30 hours. We recommend a cell density of approximately 55,000–80,000 cells/cm^2 during the transfection. For example, use approximately 4–6 × 10^6 cells for a T75 flask.

4.B. Preparing Cells with NanoBRET™ Tracer K-5 Reagent

1. Remove medium from flask with transfected HEK293 cells via aspiration, trypsinize and allow cells to dissociate from the flask.
2. Neutralize trypsin using medium containing serum (e.g., Cell Culture Medium) and centrifuge at 200 × *g* for 5 minutes to pellet the cells.
3. Aspirate medium and resuspend cells using prewarmed Assay Medium.
4. Adjust the density to 2 × 10^5 cells/ml in Assay Medium.
5. Dispense 85µl per well of cell suspension into white, 96-well NBS plates. Periodically mix cells to avoid settling in the tube.

   Optional: Dispense 90µl of cell suspension per well in triplicate as no-tracer control samples for background correction.
6. Prepare Complete 20X NanoBRET™ Tracer K-5 Reagent.
   a. First, prepare a 100X solution of NanoBRET™ Tracer K-5 in 100% DMSO. For target engagement assays for BTK-NanoLuc®, we recommend a 100X tracer concentration of 100µM for a final concentration of 1µM tracer as a starting point. Higher tracer concentrations may increase assay window but reduce sensitivity. Therefore, you may need to optimize the tracer concentration. See Figures 4 and 5 for example data.
   b. Add 4 parts of Tracer Dilution Buffer to 1 part of 100X NanoBRET™ tracer to generate Complete 20X NanoBRET™ Tracer Reagent.

   Note: Because the Tracer Dilution Buffer is viscous, slowly dispense both the Tracer Dilution Buffer and the Complete 20X NanoBRET™ Tracer Reagent. For alternate tracer preparation protocols, see Section 7.C.
7. Dispense 5µl of Complete 20X NanoBRET™ Tracer Reagent per well to cells. Mix the 96-well plate on an orbital shaker for 15 seconds at 700rpm. **Note:** Plate mixing may need to be optimized on different orbital shakers.

   Optional: Prepare a separate set of samples without tracer for optional background correction steps.

4.C. Adding Test Compounds

1. Prepare serially diluted test compound at 1,000X final concentration in 100% DMSO. Then dilute 1,000X test compound to 10X final concentration in Assay Medium.
2. Add 10µl of 10X serially diluted test compound per well of 96-well plates containing cells with 1X NanoBRET™ Tracer Reagent. Thoroughly mix plate on an orbital shaker for 15 seconds at 700rpm. **Note:** Plate mixing may need to be optimized on different orbital shakers.
3. Incubate the plate at 37°C, 5% CO_2 for 2 hours. Equilibrate plate to room temperature for ~15 minutes, then proceed to NanoBRET™ Assay Protocol, Section 4.D.

   Note: Depending on the permeability and binding characteristics of the test compound, incubation times with test compound may require optimization by the end user.

4.D. NanoBRET™ Assay Protocol

1. Remove plate from incubator and equilibrate to room temperature for 15 minutes.
2. Prepare 3X Complete Substrate plus Inhibitor Solution in Assay Medium (Opti-MEM® I Reduced Serum Medium, no phenol red) just before measuring BRET. This solution consists of a 1:166 dilution of NanoBRET™ Nano-Glo® Substrate plus a 1:500 dilution of Extracellular NanoLuc® Inhibitor in Assay Medium. For a 96-well plate, mix 30µl of NanoBRET™ Nano-Glo® Substrate, 10µl of Extracellular NanoLuc® Inhibitor and 4,960µl of Assay Medium to produce 5ml of 3X Complete Substrate plus Inhibitor Solution. Mix gently by inversion 5–10 times in a conical tube. (The final concentration of Extracellular NanoLuc® Inhibitor in the 3X solution is 60µM, for a working concentration of 20µM.)

   Note: Use 3X Complete Substrate plus Inhibitor Solution within 2 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).

   Note: We recommend measuring BRET within 10 minutes after adding NanoBRET™ Nano-Glo® Substrate plus Extracellular NanoLuc® Inhibitor Solution. You can measure BRET for up to 2 hours, but there will be some loss of luminescent signal.

4.E. 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. Convert raw BRET units to milliBRET units (mBU) by multiplying each raw BRET value by 1,000.

   NanoBRET™ ratio equation:

   $$\text{BRET Ratio} = (\text{Acceptor}_{\text{sample}} \div \text{Donor}_{\text{sample}}) \times 1{,}000$$

   NanoBRET™ ratio equation, including optional background correction:

   $$\text{BRET Ratio} = [(\text{Acceptor}_{\text{sample}} \div \text{Donor}_{\text{sample}}) - (\text{Acceptor}_{\text{no-tracer control}} \div \text{Donor}_{\text{no-tracer control}})] \times 1{,}000$$

4.F. BRET Data Generated using the BTK-NanoLuc® Fusion Vector

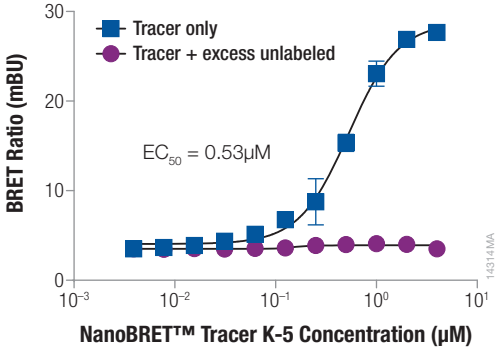
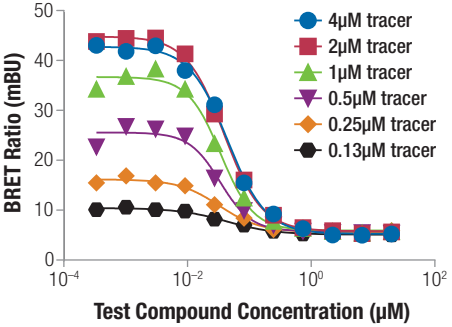


Figure 4. Apparent intracellular NanoBRET™ tracer affinity in HEK293 cells transiently expressing BTK-NanoLuc® fusion protein. HEK293 cells expressing BTK-NanoLuc® fusion protein were resuspended in Assay Medium, seeded into 96-well plates and mixed with increasing concentrations of NanoBRET™ Tracer K-5. Cells were treated with an excess of unlabeled compound as a competitive inhibitor for 2 hours before adding 3X Complete Substrate plus Inhibitor Solution. BRET measurements were made on a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Raw BRET ratios were then converted to milliBRET units (mBU) and plotted vs. NanoBRET™ tracer concentration to determine apparent intracellular affinity of the tracer.



Tracer Concentration (µM)	4	2	1	0.5	0.25	0.13
IC_{50}	0.045	0.039	**0.034**	0.034	0.033	0.043

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Figure 5. NanoBRET™ tracer competition in transiently transfected HEK293 cells expressing BTK-NanoLuc® fusion protein. HEK293 cells expressing BTK-NanoLuc® fusion protein were resuspended in Assay Medium, seeded into 96-well plates and mixed with various concentrations of NanoBRET™ Tracer K-5. Cells were treated with varying concentrations of unlabeled compound as a competitive inhibitor for 2 hours before adding 3X Complete Substrate plus Inhibitor Solution. BRET was measured using a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Raw BRET ratios were then converted to milliBRET units (mBU) and plotted vs. unmodified test compound concentration to determine apparent intracellular affinity of the unmodified test compound at each concentration of tracer. If you are using NanoBRET™ TE Intracellular Kinase Assay with BTK-NanoLuc® for the first time, our recommended concentration of 1µM NanoBRET™ Tracer K-5 is shown in bold. Note that lower tracer concentrations result in more accurate estimation of intracellular compound affinity but a lower assay window. The use of a lower concentration of tracer may be more accurate when quantifying intracellular compound affinity. See Section 5 for additional approaches to improve quantitative analysis of test compound affinity.

5. Approaches to Improving Quantitative Analysis of Test Compound Affinity

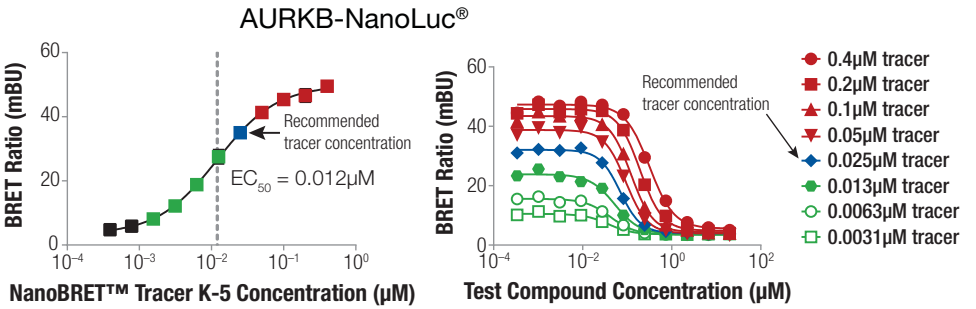
The NanoBRET™ TE Assay allows you to evaluate the affinity of a test compound for a target of interest inside living cells. However, like other competitive binding assays, the apparent affinity of a test compound can be affected by the amount of the NanoBRET™ tracer that is used in the assay. Because the tracer and test compound both compete for binding to the target NanoLuc® fusion, increasing concentrations of NanoBRET™ tracer can shift the apparent IC_{50} value of the test drug to higher concentrations. This relationship becomes most significant when the concentration of tracer is in excess of its apparent intracellular affinity for the target NanoLuc® fusion.

If you are a new user of the NanoBRET™ TE Assay, we suggest that you measure the affinity of test compounds at the recommended concentration of tracer, which is usually a sub-saturating dose between the EC_{50} and EC_{80} of the tracer. This provides a good starting point for the rank-ordering of test compound affinity for the target-NanoLuc® fusion.

Once familiar with the NanoBRET™ TE Assay, you may choose to further optimize the tracer concentration to achieve a more quantitative analysis of test compound affinity. With careful choice of the NanoBRET™ tracer concentration, the NanoBRET™ TE Assay allows you to achieve a more quantitative determination of test compound affinity that approaches the apparent intracellular affinity constant (intracellular K_i) for the target NanoLuc® fusion. To improve quantitation of test compound affinity, choose a tracer concentration that is at or below the EC_{50} of the tracer for the target NanoLuc® fusion. An example of this approach is provided in Figure 6 for a target that can be saturated when titrated with NanoBRET, as shown in the NanoBRET dose response curve. (Figure 6, Panel A). Provided that the assay window is still adequate (ideally assay window > 2), this approach will produce an IC_{50} for the test compound that is independent of the concentration of NanoBRET™ tracer used in the experiment. Due to assay window constraints when choosing a tracer concentration, this more quantitative approach is reserved only for the High Window and some Medium Window assays (see Table 2 and Section 7.A), where the use of tracer concentrations at or below the EC_{50} still provides an adequate assay window.

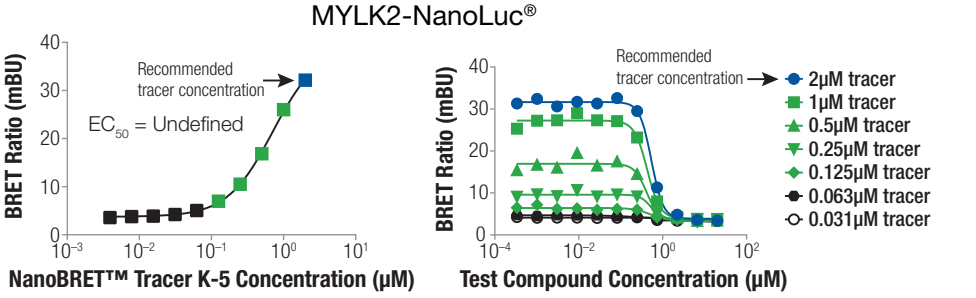
Due to solubility limits of the tracer, some targets cannot be saturated by the tracer under live-cell assay conditions. In these cases (for an example, see Figure 6, Panel B), the apparent IC_{50} value of the test compound may appear insensitive to tracer concentration. Thus, the recommended tracer concentration is already a good choice for approaching quantitative analysis.

A.



Tracer Concentration (µM)	0.4	0.2	0.1	0.05	0.025	0.013	0.0063	0.0031
Apparent IC_{50}	0.32	0.19	0.12	0.093	**0.069**	0.052	0.042	0.038
Description	Right shifted				Recommended concentration	Quantitative analysis		

B.



Tracer Concentration (µM)	2	1	0.5	0.25	0.125	0.063	0.031
Apparent IC_{50}	**0.54**	0.43	0.41	0.52	0.52	Poor fit	Poor fit
Description	Recommended concentration	Quantitative analysis				Low assay window	

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Figure 6. Approaches to improving quantitation of intracellular compound affinity using NanoBRET™ TE. Examples of tracer affinity and apparent intracellular compound affinity for a target with strong tracer affinity (**Panel A**) and a target with weak tracer affinity (**Panel B**) are provided. HEK293 cells expressing individual NanoLuc® Kinase fusions were resuspended in Assay Medium and seeded into 96-well plates. Cells were treated with various concentrations of NanoBRET™ Tracer K-5 and unlabeled compound as a competitive inhibitor and incubated for 2 hours before adding 3X Complete Substrate plus Inhibitor Solution. BRET was measured using a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Raw BRET ratios were then converted to milliBRET units (mBU) and plotted vs. NanoBRET™ Tracer K-5 concentration to determine apparent intracellular affinity of the tracer or unlabeled compound.

6. Troubleshooting

Symptoms	Causes and Comments
NanoBRET™ signal without test compound is weak or close to instrument background	Tracer was adsorbed to plasticware surface. We recommend using nonbinding surface plates. Use polypropylene materials to minimize tracer adsorption and avoid using polystyrene.
	Suboptimal tracer concentration or preparation. Consider optimizing the concentration of tracer for your experiment. Consult Section 7.C for tracer preparation options. NanoBRET™ Tracer K-5 will precipitate at concentrations above 4µM in Assay Medium or other aqueous environments.
	Instrument was set up improperly. Use the correct filters for donor wavelength (450nm) and acceptor wavelength (590nm) on your instrument to accurately measure NanoBRET™ signals.
	Low protein expression levels. To ensure that the NanoLuc® fusion protein is expressed at appropriate levels, compare the donor (450nm) and acceptor (610nm) luminescence to the background signal in the absence of cells expressing NanoLuc® luciferase but in the presence of the NanoBRET™ Nano-Glo® Substrate. Both donor and acceptor luminescence values should be significantly above the background from the substrate. Optimize transfection conditions to improve expression of the NanoLuc® fusion protein.
Observed IC_{50} value is right-shifted compared to expected value	During correct execution of the assay, cell-based analyses of target engagement may result in right-shifted pharmacology relative to that observed in a biochemical assay due to myriad cellular factors. These include permeability, the presence of endogenous metabolites, target activation state, or the presence of intracellular complexes. Moreover, target engagement parameters for full-length targets in a cellular context may differ from that of truncated domains commonly used in biochemical assays.
	The concentration of the NanoBRET™ tracer may affect the observed IC_{50} value. Carefully select tracer concentration (see Section 5). Determine a more accurate compound IC_{50} value by optimizing the tracer concentration.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Donor or acceptor luminescence increases or decreases when tracer is added	This phenomenon is common but generally does not affect the assay. Figure 7 demonstrates representative data showing raw luminescence from donor (450nm) and acceptor (610nm) channels when NanoBRET™ Tracer K-5 is titrated. BRET that occurs between the NanoLuc® fusion protein and fluorescent tracer may result in a dose-dependent increase in acceptor luminescence with a corresponding decrease in donor luminescence. The effect of BRET on donor and acceptor luminescence may vary, depending on the target and tracer used. Ratiometric BRET analysis mitigates influence of fluctuations in raw luminescence from NanoLuc® luciferase.

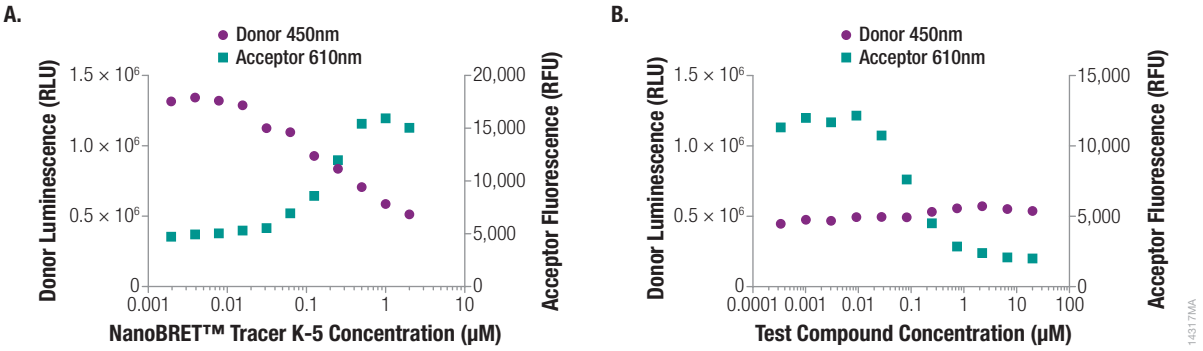

Figure 7. Potential effects of raw luminescence on donor and acceptor emission in the NanoBRET™ TE Assay in a tracer dose response experiment or compound dose response experiment. Panel A. The tracer dose response experiment for the BTK-NanoLuc® fusion protein was carried out as described in Figure 4, using a dose response of tracer in the presence or absence of 20µM unlabeled compound as competitor. **Panel B.** The compound dose response experiment for the BTK-NanoLuc® fusion protein was carried out as described in Figure 5, using a fixed tracer concentration of 1µM tracer and a dose response of the unlabeled compound.

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7. Appendix

7.A. Representative Target Engagement Data and Assay Capabilities

The NanoBRET™ TE Intracellular Kinase Assay, K-5, is compatible with a diverse set of intracellular kinases. A list of compatible kinase-NanoLuc® fusion vectors is provided in Section 7.H. The affinity of the K-5 tracer varies among these compatible kinases and the K-5 tracer concentration should be adjusted accordingly for optimal assay performance.

For each K-5 compatible kinase-NanoLuc® fusion vector we provide the K-5 tracer affinity and recommended K-5 tracer concentration in an application note, included as a link on the kinase-NanoLuc® fusion vector web page. Application notes are also listed in the Kinase Vector Data Selector table on the NanoBRET™ TE Intracellular Kinase Assay web page: **https://www.promega.com/products/cell-signaling/kinase-target-engagement/nanobret-te-intracellular-kinase-assay/**

When using this NanoBRET™ TE Intracellular Kinase Assay, K-5, protocol with a compatible kinase other than the control kinase NanoLuc®-BTK Vector, there are two changes you need to make to the protocol: 1) In section 4.A., Step 6, prepare the lipid:DNA complexes using your kinase NanoLuc® fusion vector of interest, instead of the control NanoLuc®-BTK vector; 2) In section 4.B. Step 6, prepare 100X tracer using 100X the recommended tracer concentration listed in the application note. Follow the remaining protocol steps as written.

For a more accurate estimate of intracellular compound affinity, it's also possible to use a lower tracer concentration than recommended. Approaches to achieve a more quantitative measurement of compound affinity are provided in Section 5 of this Technical Manual, as well as the Application Note.

One other difference that may be observed when using the NanoBRET™ TE Kinase Assay, K-5, with a compatible kinase fusion vector is the assay window. Due to the distance and geometry components of BRET, the assay window may not be the same as observed with the control NanoLuc®-BTK vector. This can influence the overall assay capabilities. Since the NanoBRET™ Assay window may vary among compatible kinases, we have organized the assays into groupings for a particular kinase target given the assay window (Table 2).

Table 2. NanoBRET™ TE Assay Capabilities.

Assay Category	Assay Window (AW)[1]	Assay Capabilities
High Window	AW ≥ 3.0	Multiple-dose compound profiling to determine IC_{50} at a fixed concentration of tracer in 96- or 384-well format. Low- to medium-throughput single-dose profiling at fixed tracer concentration. Excellent candidate for further miniaturization with optimization.
Medium Window	3.0 > AW ≥ 2.0	Multiple-dose compound profiling to determine IC_{50} at fixed concentration of tracer in 96-well format. Possible candidate for scale-down to 384-well format with optimization by the end user. Possible candidate for low to medium throughput single-dose profiling at a fixed tracer concentration with optimization.
Low Window	2.0 > AW ≥ 1.6	Multiple-dose compound profiling to determine IC_{50} at fixed concentration of tracer in 96-well format.

[1]Assay window is the raw fold change in the BRET ratio observed at the recommended concentration of tracer compared to the BRET ratio in the presence of a saturating dose of unlabeled compound.

7.A. Representative Target Engagement Data and Assay Capabilities (continued)

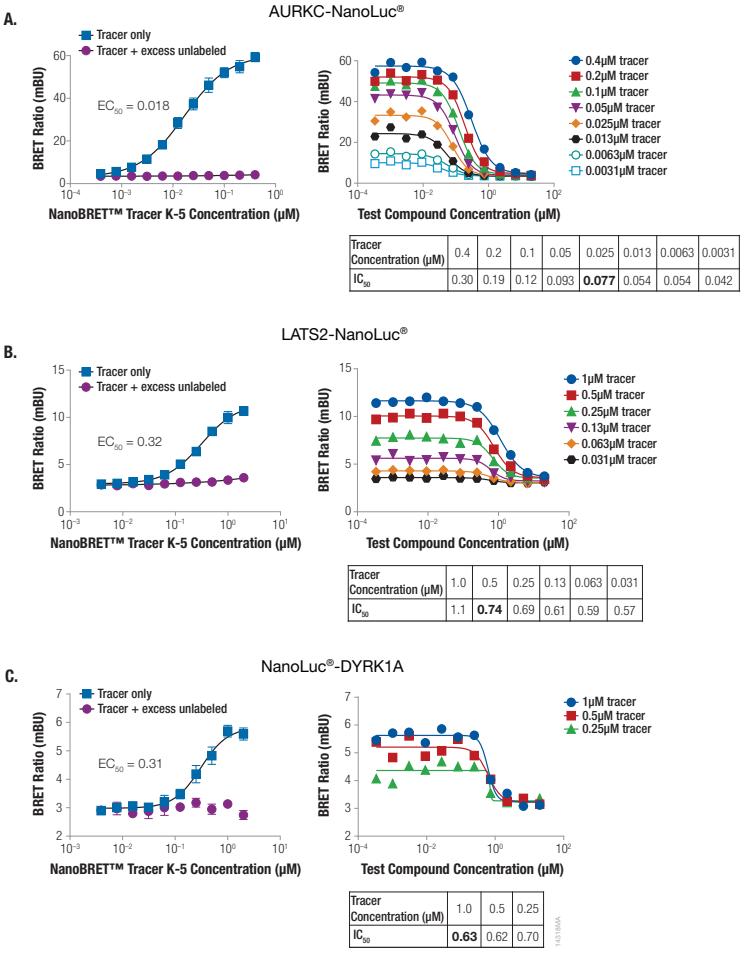


Figure 8. NanoBRET™ Tracer K-5 affinity and competition in HEK293 cells transiently expressing NanoLuc® Kinase fusion proteins. Examples of High Window (**Panel A**), Medium Window (**Panel B**) and Low Window (**Panel C**) assays are provided. Discussion of each category are provided in Table 2. HEK293 cells expressing individual NanoLuc® Kinase fusions were resuspended in Assay Medium and seeded into 96-well plates. Cells were treated with various concentrations of NanoBRET™ tracer and unlabeled test compound as a competitive inhibitor and incubated for 2 hours before adding 3X Complete Substrate plus Inhibitor Solution. BRET was measured using a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Raw BRET ratios were then converted to milliBRET units (mBU) and plotted vs. tracer concentration to determine apparent intracellular affinity of the tracer or unlabeled test compound. The IC_{50} value generated using the recommended concentration of tracer for each target is shown in bold.

7.B. Performing the NanoBRET™ TE Assay in Adherent Format

Due to the chemical properties of NanoBRET™ tracers, we generally recommend that you follow the NanoBRET™ TE protocol described in Section 4, which uses a Non-Binding Surface (NBS) assay plate. However, you may wish to perform the assay under conditions that are closer to those used in other cell-based phenotypic assays, for example, using adhered cells in a tissue culture (TC)-treated assay plate. In addition to the NBS format protocol described in Section 4, the NanoBRET™ TE Assay using NanoBRET™ Tracer K-5 can be performed in an alternative format using adherent cells in a TC-treated assay plate (ADH format). Performing the assay in ADH format requires a few subtle modifications to the protocol described in Section 4. In addition, we recommend that you revalidate the assay in ADH format for each kinase target of interest by re-evaluating both the affinity of the tracer and the effect of tracer concentration on test compound IC_{50}. This allows you to choose an optimum tracer concentration for evaluating compound IC_{50} to the target of interest in ADH format, which might be different than that recommended for use in NBS format. Protocol adjustments for ADH format and example validation data for BTK-NanoLuc® fusion protein can be found below.

Note: Due to unique chemical properties, not all NanoBRET™ tracers are compatible with ADH format. For specific NanoBRET™ tracer products, compatibility with ADH format will be specifically noted in the corresponding technical manual.

To perform the NanoBRET™ TE Assay in ADH format using HEK293 cells, we recommend a few subtle adjustments to the NBS format protocol described in Section 4. Protocol adjustments occur in the following sections:

- Section 4. Materials to be Supplied by the User
- Section 4.A. Transient Transfection of HEK293 Cells
- Section 4.B. Preparing Cells with NanoBRET™ Tracer Reagent

Note: Sections 4.C–E can be used with ADH assay format without modification. To use adherent cells, follow the steps here in Section 7.B, then Sections 4.C–E to complete the protocol.

Alternative Materials to be Supplied by the User for ADH Format

- assay plates: white, tissue cultured-treated (TC) 96-well (Corning Cat.# 3917) or 384-well (Corning Cat.# 3570)
- vacuum aspirator with 8-channel adapter (Corning Cat.# 4930 and 4931)

Alternative Protocol for Transient Transfection of HEK293 Cells for ADH Format

1. Cultivate HEK293 cells (or desired cell type) appropriately prior to assay. **Note:** If other cell types are used, optimize the transfection conditions.
2. Remove medium from cell flask by aspiration, trypsinize and allow cells to dissociate from the flask.
3. Neutralize trypsin using Cell Culture Medium and centrifuge at 200 × *g* for 5 minutes to pellet cells.
4. Aspirate medium and resuspend cells in Cell Culture Medium.
5. Adjust density to 2×10^5 cells/ml using Cell Culture Medium.

7.B. Performing the NanoBRET™ TE Assay in Adherent Format (continued)

Alternative Protocol for Transient Transfection of HEK293 Cells for ADH format (continued)

6. If HEK293 cells are used, prepare lipid:DNA complexes as follows:
    a. Prepare a 10µg/ml solution of DNA in Assay Medium that consists of the following ratios: 9.0µg/ml of Transfection Carrier DNA, 1.0µg/ml of NanoLuc® fusion DNA and 1ml of Assay Medium. To accurately dilute the NanoLuc® fusion DNA, serially dilute the fusion vector with Transfection Carrier DNA to maintain the same amount of DNA (e.g., 10µg).
    b. Mix thoroughly.
    c. Add 30µl of FuGENE® HD Transfection Reagent into each milliliter of DNA mixture to form lipid:DNA complex. Ensure that the FuGENE® HD Transfection Reagent does not touch the plastic side of the tube; pipet directly into the liquid in the tube.
    d. Mix by inversion 5–10 times.
    e. Incubate at ambient temperature for 20 minutes to allow complexes to form.
7. In a sterile, conical tube, mix 1 part of lipid:DNA complex (e.g., 1ml) with 20 parts of HEK293 cells (e.g., 20ml) in suspension at 2×10^5 cells/ml. Mix gently by inversion 5 times.

    Note: Larger or smaller bulk transfections should be scaled accordingly, using this 20:1 cells to lipid:DNA complex ratio.
8. Dispense 100µl cells + lipid:DNA complex into a sterile, tissue-culture treated 96-well plate, and incubate at least 20 hours to allow expression. We recommend a cell density of approximately 55,000 to 80,000 cells/cm^2 during the transfection (for example, use approximately 20,000 cells/well for a 96-well Corning Cat. #3917 assay plate).

Alternative Protocol for Preparing Cells with NanoBRET™ Tracer Reagent for ADH Format

1. Gently remove medium from the assay plate containing transfected HEK293 cells via aspiration.
2. Dispense 85µl per well of Assay Medium into the assay plate.

    Optional: Dispense 90µl of cell suspension per well in triplicate as no-tracer control samples for background correction.
3. Prepare Complete 20X NanoBRET™ Tracer Reagent.
    a. First, prepare a 100X solution of NanoBRET™ Tracer K-5 in 100% DMSO. Higher tracer concentrations may increase assay window but reduce sensitivity. Therefore, you may need to optimize the tracer concentration. See Figures 4 and 5 for example data. For target engagement assays for BTK-NanoLuc®, we recommend a 100X tracer concentration of 25µM for a final concentration of 0.25µM tracer as a starting point.
    b. Mix 1 part of 100X tracer with 4 parts Tracer Dilution Buffer to generate Complete 20X NanoBRET™ Tracer Reagent.

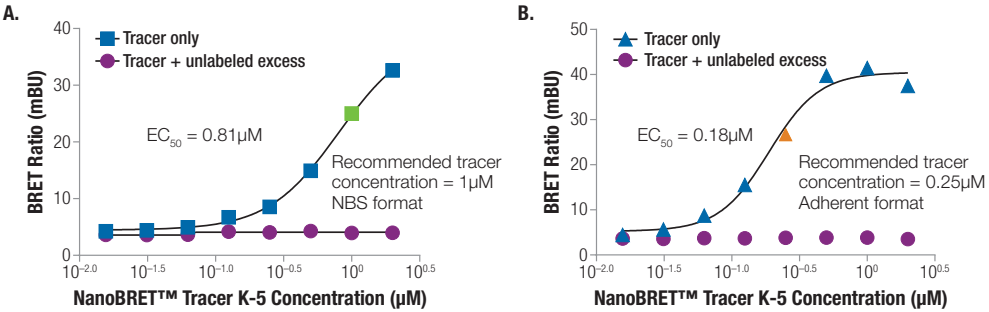
    Note: Because the Tracer Dilution Buffer is viscous, slowly dispense both the Tracer Dilution Buffer and the Complete 20X NanoBRET™ Tracer Reagent.

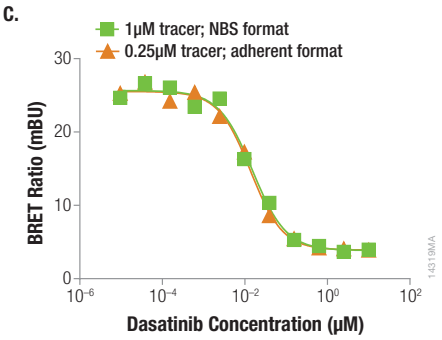
7.B. Performing the NanoBRET™ TE Assay in Adherent Format (continued)

4. Dispense 5µl of Complete 20X NanoBRET™ Tracer Reagent per well to cells. Mix the 96-well plate on an orbital shaker for 15 seconds at 700rpm. **Note:** Plate mixing may need to be optimized on different orbital shakers.

   Optional: Prepare a separate set of samples without tracer for optional background correction steps.

5. Follow the protocol steps in Sections 4.C–E to complete preparation of adherent cells.





Tracer Concentration (µM)	NBS	Adherent
IC_{50}	0.015	0.014

Figure 9. Comparison of NanoBRET™ assay validation for BTK-NanoLuc® fusion protein in NBS (non-binding surface) versus ADH (adherent) format. HEK293 cells expressing BTK-NanoLuc® fusion protein were assayed in either NBS format by the recommended protocol (Section 4) or in ADH format. Cells were treated with increasing concentrations of NanoBRET™ Tracer K-5 with an excess of unlabeled compound (20µM) as a competitive inhibitor for 2 hours before adding 3X Complete Substrate plus Inhibitor Solution. BRET measurements were made on a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Raw BRET ratios were then converted to milliBRET units (mBU) and plotted vs. tracer concentration to determine apparent intracellular affinity of the tracer in either NBS format (**Panel A**) or ADH format (**Panel B**). Recommended tracer concentrations for compound IC_{50} determination are highlighted in green (NBS format) or orange (ADH format), respectively. **Panel C** shows affinity of test drug dasatinib, determined in both NBS and ADH format at the recommended tracer concentrations, with both formats yielding comparable IC_{50} values.

7.C. Modifications to Tracer Preparation Workflow to Adjust the Final DMSO Concentration

Because the NanoBRET™ TE assay uses live, intact cells, it is important to consider the final concentration of DMSO or other possible vehicle solvents in the assay well. DMSO is introduced into the assay from both the NanoBRET™ Tracer Reagent and (possibly) from the test compound solution. When performing the standard NBS protocol as described in Section 4, the tracer prepared from a 100X stock in DMSO contributes 1% (v/v) DMSO and a test compound prepared from a 1,000X stock solution in DMSO contributes 0.1% (v/v) DMSO to the assay solution for a total final concentration of 1.1% (v/v) DMSO during the compound incubation step. We recommend that you stay below this level of DMSO to avoid toxicity effects.

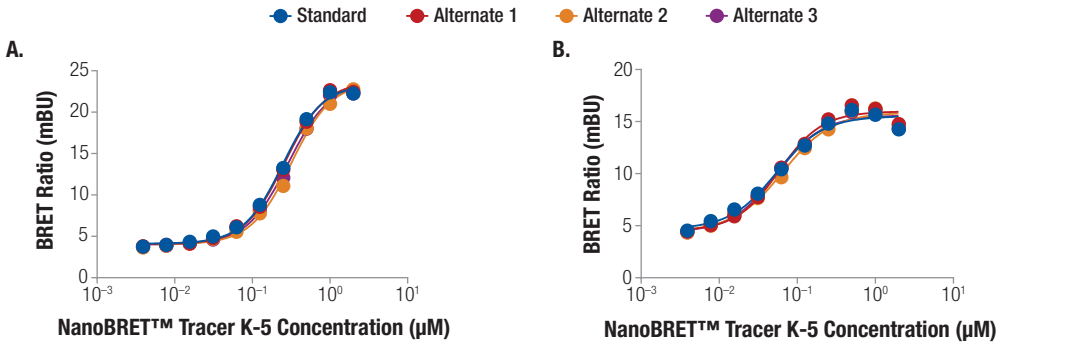
If you are limited by compound solubility in DMSO or have compound stock solutions prepared at lower than 1,000X in DMSO, it is possible to adjust the preparation of NanoBRET™ Tracer K-5 in order to reduce its contribution of DMSO to the assay solution. Specifically, NanoBRET™ Tracer K-5 can be prepared at up to an 800X stock solution in DMSO before diluting to the 20X working solution with Tracer Dilution Buffer, which will significantly reduce the contribution of DMSO to the assay while still maintaining adequate assay performance. See Table 3 and Figure 10 for examples of tracer preparation options and the amount of DMSO contributed to the assay solution.

Note: This adjustment to the tracer preparation protocol has not been globally evaluated for all kinases compatible with NanoBRET™ Tracer K-5. We recommend that you re-evaluate the affinity of the tracer for the kinase target of interest to ensure adequate assay performance compared to the standard protocol. Comparable apparent tracer affinity and assay window using the alternate formulation compared to the standard protocol would indicate adequate assay performance. Right-shifting of apparent tracer affinity or reduction in assay window compared to the standard protocol would indicate a negative change in assay performance.

Table 3. Contribution of DMSO using Alternate NanoBRET™ Tracer Preparation Protocols.

Protocol	Concentrated Tracer Stock	Working Tracer Stock	Preparation of Working Tracer Stock	DMSO Contributed to Assay
Standard	100X in DMSO	20X in TDB[1]	1 part concentrated + 4 parts TDB[1]	1% (v/v)
Alternate 1	200X in DMSO	20X in TDB[1]	1 part concentrated + 9 parts TDB[1]	0.5% (v/v)
Alternate 2	400X in DMSO	20X in TDB[1]	1 part concentrated + 19 parts TDB[1]	0.25% (v/v)
Alternate 3	800X in DMSO	20X in TDB[1]	1 part concentrated + 39 parts TDB[1]	0.125% (v/v)

[1]TDB = Tracer Dilution Buffer



	Standard	Alternate 1	Alternate 2	Alternate 3
EC_{50} (µM)	0.25	0.26	0.32	0.29

	Standard	Alternate 1	Alternate 2	Alternate 3
EC_{50} (µM)	0.054	0.058	0.065	0.055

14320MA

Figure 10. Comparison of NanoBRET™ Tracer K-5 affinity using alternate tracer preparation protocols. See Table 3 for details on preparation procedures for the standard protocol versus protocol alternates 1 through 3. HEK293 cells expressing BTK-NanoLuc® fusion protein (**Panel A**) or PTK2-NanoLuc® fusion protein (**Panel B**) were resuspended in Assay Medium, seeded into 96-well plates and mixed with increasing concentrations of tracer. Cells were incubated for 2 hours before adding 3X Complete Substrate plus Inhibitor Solution. BRET measurements were made on a GloMax® Discover System equipped with NanoBRET™ 618 filters (donor 450nm/8nm BP and acceptor 600nm LP). Raw BRET ratios were converted to milliBRET units (mBU) and plotted vs. tracer concentration to determine apparent intracellular affinity of the tracer.

7.D. Preparing Stable Cell Lines Expressing NanoLuc® Fusion Proteins

The NanoLuc® expression vectors use relatively strong constitutive promoters. To avoid overexpression in stable cell lines, we recommend the use of attenuated promoters for appropriate expression of the NanoLuc® fusion protein. Please contact Custom Assay Services for custom preparation of stable cell lines expressing NanoLuc® luciferase fusion proteins at: **www.promega.com/products/custom-assay-services/**

7.E. Composition of Buffers and Solutions

Cell Culture Medium

90% DMEM (Thermo Fisher Cat #11995-065, Seradigm Cat.# 1500-050)

10% fetal bovine serum (FBS; 9/17 Cat.# SH30070.03)

Assay Medium

100% Opti-MEM® I Reduced Serum Medium, no phenol red (Life Technologies Cat.# 11058-021)

7.F. 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. *Nature Comm.* **6**, 10091.
3. Anthropological Genetics: Theory, Methods and Applications, Michael H. Crawford, *ed.* (2006) University of Cambridge Press.

7.G. Extinction Coefficient of NanoBRET™ Tracer K-5

NanoBRET™ Tracer K-5 uses the NanoBRET™ 590 fluorophore. The concentration of NanoBRET™ Tracer K-5 was determined using an extinction coefficient of 83,000 M^{-1} cm^{-1} at 590nm. See Table 10.1 in reference for details (3).

7.H. Related Products

NanoBRET™ Target Engagement Assays and Reagents

Product	Size	Cat.#
NanoBRET™ TE Intracellular Kinase Assay, K-4	100 assays	N2520
	1,000 assays	N2521
NanoBRET™ TE Intracellular Kinase Detection Reagents, K-4	10,000 assays	N2540
NanoBRET™ TE Intracellular HDAC Assay	100 assays	N2080
	1,000 assays	N2081
NanoBRET™ TE Intracellular HDAC Detection Reagents	10,000 assays	N2090
NanoBRET™ TE Intracellular BET BRD Assay	100 assays	N2130
	1,000 assays	N2131
NanoBRET™ TE Intracellular BET BRD Detection Reagents	10,000 assays	N2140
Intracellular TE Nano-Glo® Substrate/Inhibitor	1,000 assays	N2160
	10,000 assays	N2161
Tracer Dilution Buffer	50ml	N2191
Transfection Carrier DNA	100µg	E4881

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7.H. Related Products (continued)

NanoBRET™ Tracer K-4 Compatible Kinase-NanoLuc® Fusion Vectors

Product	Size	Cat.#
NanoLuc®-ABL1 Fusion Vector	20µg	NV1011
BMX-NanoLuc® Fusion Vector	20µg	NV1101
CSF1R-NanoLuc® Fusion Vector	20µg	NV1161
CSK-NanoLuc® Fusion Vector	20µg	NV1171
DDR2-NanoLuc® Fusion Vector	20µg	NV1201
EPHA1-NanoLuc® Fusion Vector	20µg	NV1221
EPHA2-NanoLuc® Fusion Vector	20µg	NV1231
EPHA4-NanoLuc® Fusion Vector	20µg	NV1241
EPHA5-NanoLuc® Fusion Vector	20µg	NV1251
EPHA8-NanoLuc® Fusion Vector	20µg	NV1281
EPHB2-NanoLuc® Fusion Vector	20µg	NV1291
EPHB3-NanoLuc® Fusion Vector	20µg	NV1301
EPHB4-NanoLuc® Fusion Vector	20µg	NV1311
NanoLuc®-FGR Fusion Vector	20µg	NV1381
FRK-NanoLuc® Fusion Vector	20µg	NV1401
FYN-NanoLuc® Fusion Vector	20µg	NV1411
KIT-NanoLuc® Fusion Vector	20µg	NV1491
LCK-NanoLuc® Fusion Vector	20µg	NV1521
LIMK2-NanoLuc® Fusion Vector	20µg	NV1531
LYN-NanoLuc® Fusion Vector	20µg	NV1551
NanoLuc®-MAPK11 Fusion Vector	20µg	NV1651
MAPK14-NanoLuc® Fusion Vector	20µg	NV1661
PTK6-NanoLuc® Fusion Vector	20µg	NV1941
NanoLuc®-RIPK2 Fusion Vector	20µg	NV1971
NanoLuc®-SIK1 Fusion Vector	20µg	NV2031
NanoLuc®-SIK3 Fusion Vector	20µg	NV2041
NanoLuc®-SNF1LK2 Fusion Vector	20µg	NV2061
SRC-NanoLuc® Fusion Vector	20µg	NV2071
NanoLuc®-TEC Fusion Vector	20µg	NV2141
NanoLuc®-TESK1 Fusion Vector	20µg	NV2161
TXK-NanoLuc® Fusion Vector	20µg	NV2201
YES1-NanoLuc® Fusion Vector	20µg	NV2241

7.H. Related Products (continued)

NanoBRET™ Tracer K-5 Compatible Kinase-NanoLuc® Fusion Vectors

Product	Size	Cat.#
NanoLuc®-AAK1 Fusion Vector	20µg	NV1001
ACVR1B-NanoLuc® Fusion Vector	20µg	NV1021
AKT2-NanoLuc® Fusion Vector	20µg	NV1031
AURKA-NanoLuc® Fusion Vector	20µg	NV1041
AURKB-NanoLuc® Fusion Vector	20µg	NV1051
AURKC-NanoLuc® Fusion Vector	20µg	NV1061
AXL-NanoLuc® Fusion Vector	20µg	NV1071
NanoLuc®-BMP2K Fusion Vector	20µg	NV1091
NanoLuc®-BRSK2 Fusion Vector	20µg	NV1111
CDK5-NanoLuc® Fusion Vector	20µg	NV1121
NanoLuc®-CLK1 Fusion Vector	20µg	NV1131
CLK2-NanoLuc® Fusion Vector	20µg	NV1141
CLK4-NanoLuc® Fusion Vector	20µg	NV1151
NanoLuc®-CSNK1G2 Fusion Vector	20µg	NV1181
CSNK2A2-NanoLuc® Fusion Vector	20µg	NV1191
NanoLuc®-DYRK1B Fusion Vector	20µg	NV1211
EPHA6-NanoLuc® Fusion Vector	20µg	NV1261
EPHA7-NanoLuc® Fusion Vector	20µg	NV1271
ERN1-NanoLuc® Fusion Vector	20µg	NV1321
FER-NanoLuc® Fusion Vector	20µg	NV1331
FGFR1-NanoLuc® Fusion Vector	20µg	NV1341
FGFR2-NanoLuc® Fusion Vector	20µg	NV1351
FGFR3-NanoLuc® Fusion Vector	20µg	NV1361
FGFR4-NanoLuc® Fusion Vector	20µg	NV1371
FLT3-NanoLuc® Fusion Vector	20µg	NV1391
NanoLuc®-GAK Fusion Vector	20µg	NV1421
NanoLuc®-IKBKE Fusion Vector	20µg	NV1431
NanoLuc®-IRAK3 Fusion Vector	20µg	NV1441
IRAK4-NanoLuc® Fusion Vector	20µg	NV1451
NanoLuc®-ITK Fusion Vector	20µg	NV1461
JAK3-NanoLuc® Fusion Vector	20µg	NV1471
JNK3-NanoLuc® Fusion Vector	20µg	NV1481

7.H. Related Products (continued)

NanoBRET™ Tracer K-5 Compatible Kinase-NanoLuc® Fusion Vectors (continued)

Product	Size	Cat.#
LATS1-NanoLuc® Fusion Vector	20µg	NV1501
LATS2-NanoLuc® Fusion Vector	20µg	NV1511
LTK-NanoLuc® Fusion Vector	20µg	NV1541
NanoLuc®-MAP3K10 Fusion Vector	20µg	NV1561
NanoLuc®-MAP3K11 Fusion Vector	20µg	NV1571
NanoLuc®-MAP3K12 Fusion Vector	20µg	NV1581
MAP3K4-NanoLuc® Fusion Vector	20µg	NV1591
NanoLuc®-MAP3K9 Fusion Vector	20µg	NV1601
NanoLuc®-MAP4K1 Fusion Vector	20µg	NV1611
NanoLuc®-MAP4K2 Fusion Vector	20µg	NV1621
NanoLuc®-MAP4K3 Fusion Vector	20µg	NV1631
NanoLuc®-MAPK1 Fusion Vector	20µg	NV1641
NanoLuc®-MAPK3 Fusion Vector	20µg	NV1671
NanoLuc®-MAPK4 Fusion Vector	20µg	NV1681
NanoLuc®-MAPK6 Fusion Vector	20µg	NV1691
NanoLuc®-MAPK8 Fusion Vector	20µg	NV1701
NanoLuc®-MAPK9 Fusion Vector	20µg	NV1711
NanoLuc®-MARK2 Fusion Vector	20µg	NV1721
NanoLuc®-MARK4 Fusion Vector	20µg	NV1731
NanoLuc®-MELK Fusion Vector	20µg	NV1741
MET-NanoLuc® Fusion Vector	20µg	NV1751
MUSK-NanoLuc® Fusion Vector	20µg	NV1761
MYLK2-NanoLuc® Fusion Vector	20µg	NV1771
NanoLuc®-NEK2 Fusion Vector	20µg	NV1781
NanoLuc®-NEK3 Fusion Vector	20µg	NV1791
NanoLuc®-NEK9 Fusion Vector	20µg	NV1801
NTRK1-NanoLuc® Fusion Vector	20µg	NV1811
NTRK2-NanoLuc® Fusion Vector	20µg	NV1821
NanoLuc®-NUAK1 Fusion Vector	20µg	NV1831
PAK4-NanoLuc® Fusion Vector	20µg	NV1841
PAK7-NanoLuc® Fusion Vector	20µg	NV1851
NanoLuc®-PHKG1 Fusion Vector	20µg	NV1861
PKMYT1-NanoLuc® Fusion Vector	20µg	NV1871

NanoBRET™ Tracer K-5 Compatible Kinase-NanoLuc® Fusion Vectors (continued)

Product	Size	Cat.#
NanoLuc®-PLK4 Fusion Vector	20µg	NV1881
NanoLuc®-PRKAA2 Fusion Vector	20µg	NV1891
PRKACA-NanoLuc® Fusion Vector	20µg	NV1901
PRKX-NanoLuc® Fusion Vector	20µg	NV1911
NanoLuc®-PTK2 Fusion Vector	20µg	NV1921
PTK2B-NanoLuc® Fusion Vector	20µg	NV1931
RET-NanoLuc® Fusion Vector	20µg	NV1951
NanoLuc®-RIOK2 Fusion Vector	20µg	NV1961
NanoLuc®-RPS6KA1 Fusion Vector	20µg	NV1981
NanoLuc®-RPS6KA2 Fusion Vector	20µg	NV1991
NanoLuc®-RPS6KA3 Fusion Vector	20µg	NV2001
NanoLuc®-RPS6KA4 Fusion Vector	20µg	NV2011
NanoLuc®-RPS6KA6 Fusion Vector	20µg	NV2021
NanoLuc®-SLK Fusion Vector	20µg	NV2051
NanoLuc®-STK11 Fusion Vector	20µg	NV2081
NanoLuc®-STK16 Fusion Vector	20µg	NV2091
NanoLuc®-STK32B Fusion Vector	20µg	NV2101
NanoLuc®-STK33 Fusion Vector	20µg	NV2111
STK38-NanoLuc® Fusion Vector	20µg	NV2121
NanoLuc®-TBK1 Fusion Vector	20µg	NV2131
TEK-NanoLuc® Fusion Vector	20µg	NV2151
TIE1-NanoLuc® Fusion Vector	20µg	NV2171
NanoLuc®-TNK1 Fusion Vector	20µg	NV2181
TTK-NanoLuc® Fusion Vector	20µg	NV2191
NanoLuc®-ULK1 Fusion Vector	20µg	NV2211
NanoLuc®-ULK2 Fusion Vector	20µg	NV2221
WEE1-NanoLuc® Fusion Vector	20µg	NV2231

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7.H. Related Products (continued)

Kinase Activity Assays

Product	Size	Cat.#
ADP-Glo™ Kinase Assay	400 assays	V6930
	1,000 assays	V9101
	10,000 assays	V9102
	100,000 assays	V9103
ADP-Glo™ Max Assay	1,000 assays	V7001
	10,000 assays	V7002
Kinase-Glo® Luminescent Kinase Assay	10ml	V6711
	10 × 10ml	V6712
	100ml	V6713
	10 × 100ml	V6714
Kinase Selectivity Profiling System: General Panel	24 × 50 reactions	V6928
Kinase Selectivity Profiling System: TK-1	8 × 50 reactions	V6850
Kinase Selectivity Profiling System: CMGC-1	8 × 50 reactions	V6854
Kinase Selectivity Profiling System: AGC-1	8 × 50 reactions	V6858
Kinase Selectivity Profiling System: CAMK-1	8 × 50 reactions	V6932
Kinase Selectivity Profiling System: TKL-1	8 × 50 reactions	V6914
Kinase Selectivity Profiling System: STE-1	8 × 50 reactions	V6916
Kinase Selectivity Profiling System: Other/CK-1	8 × 50 reactions	V6918

Multimode Detection Instrument

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

Transfection Reagent

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

7.I. Summary of Changes

TM520 was revised 11/2017 to add three fusion vectors to 7.H. Related Products: Cat.# NV1491, NV1511 and NV1591.

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[c]Licensed under EP1295121 and EP1088233.

[d]U.S. Pat. No. 9,056,885 and other patents pending.

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

[f]U.S. Pat. Nos. 8,557,970, 8,669,103, European Pat. No. 2990478 and other patents and patents pending.

[g]Patent Pending.

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TM520 · Revised 11/17