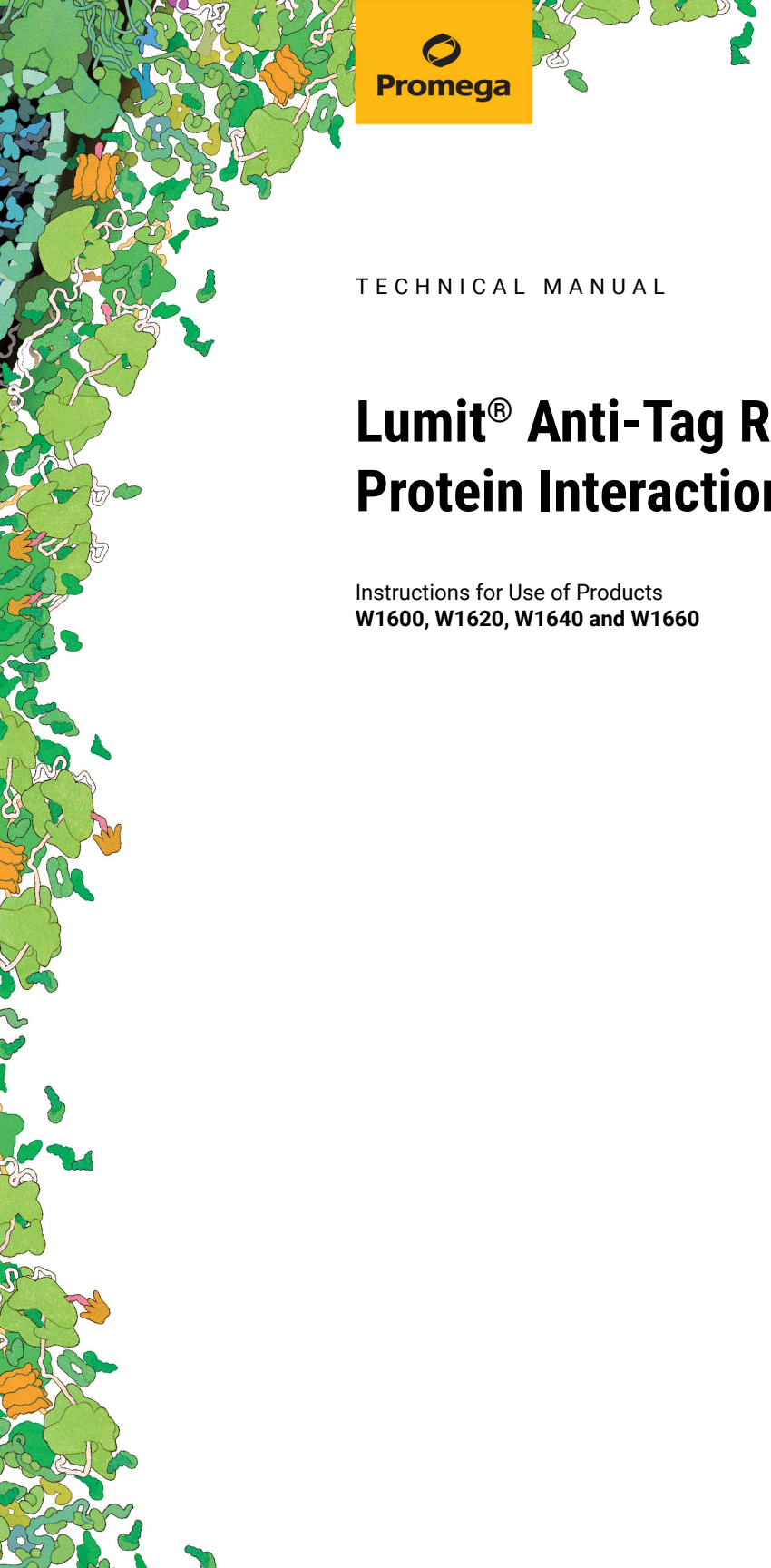

TECHNICAL MANUAL

Lumit® Anti-Tag Reagents for Protein Interactions

Instructions for Use of Products
W1600, W1620, W1640 and W1660

Revised 2/26
TM723

Lumit® Anti-Tag Reagents for Protein Interactions

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

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1. Description

Lumit® Anti-Tag Reagents for Protein Interactions[a,b,c] are homogeneous (no-wash) immunoassays to measure protein:protein or protein:small molecule interactions (Figure 1). These reagents function by combining immunodetection and NanoLuc® Binary Technology (NanoBiT). NanoBiT® luciferase is a structural complementation reporter ideal for interaction studies. The NanoBiT® system is composed of two subunits: Large BiT (LgBiT; 18kDa) and Small BiT (SmBiT; 11 amino acid peptide) that can be expressed as recombinant protein fusions or chemically conjugated to antibodies or other target proteins of interest. The LgBiT and SmBiT subunits have been optimized for stability and minimal self-association due to their weak affinity (K_d = 190µM). When LgBiT and SmBiT are brought into proximity, they form a functional enzyme that generates luminescence in the presence of a Nano-Glo® substrate.

Lumit® Anti-Tag Reagents for Protein Interactions interact with glutathione-S-transferase (GST), FLAG® or polyhistidine (6-His) tags via tag-specific antibodies chemically conjugated to LgBiT or SmBiT. Similarly, biotinylated molecules can interact with LgBiT- or SmBiT-conjugated streptavidin. When two proteins or a protein and small molecule with different tags are in proximity, the interaction can be detected using the corresponding SmBiT- and LgBiT-conjugated antibodies or streptavidin, as shown in Figure 1. The Lumit® Anti-Tag Reagents provide a biochemical immunoassay format for simple, high-throughput screening of protein:protein or protein:small molecule interactions.

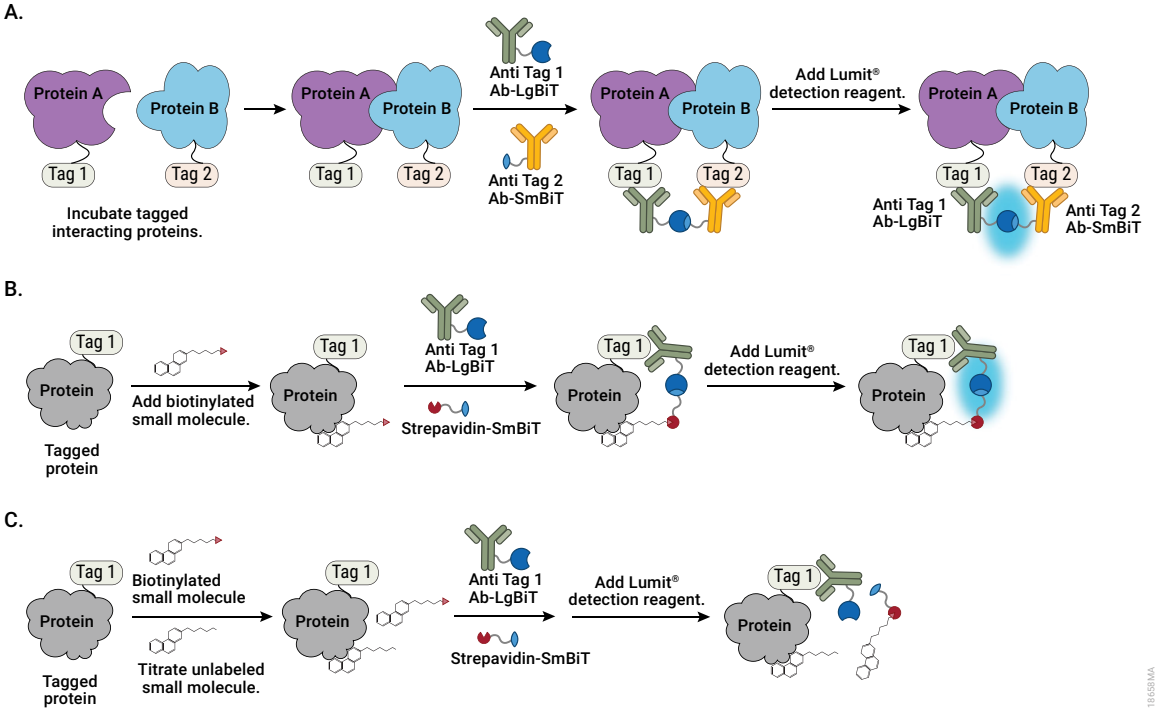

Figure 1. Panel A. Schematic of a Lumit® protein interaction immunoassay. **Panel B.** Schematic of a Lumit® small molecule interaction immunoassay. **Panel C.** Titration with unlabeled small molecule in a Lumit® small molecule interaction immunoassay.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Lumit® Anti-6His-LgBiT and Anti-6His-SmBiT	**each**	**W1600**

This bundle is sufficient to perform at least 200 reactions when used at the highest recommended concentration in 96-well plates using a 50µl final reaction volume. Includes:

- 20µl Lumit® Anti-6His-LgBiT
- 20µl Lumit® Anti-6His-SmBiT

PRODUCT	SIZE	CAT.#
Lumit® Anti-GST-LgBiT and Anti-GST-SmBiT	**each**	**W1620**

This bundle is sufficient to perform at least 200 reactions when used at the highest recommended concentration in 96-well plates using a 50µl final reaction volume. Includes:

- 20µl Lumit® Anti-GST-LgBiT
- 20µl Lumit® Anti-GST-SmBiT

PRODUCT	SIZE	CAT.#
Lumit® Anti-DYKDDDDK-LgBiT and Anti-DYKDDDDK-SmBiT	**each**	**W1640**

This bundle is sufficient to perform at least 200 reactions when used at the highest recommended concentration in 96-well plates using a 50µl final reaction volume. Includes:

- 20µl Lumit® Anti-DYKDDDDK-LgBiT
- 20µl Lumit® Anti-DYKDDDDK-SmBiT

PRODUCT	SIZE	CAT.#
Lumit® Streptavidin-LgBiT and Streptavidin-SmBiT	**each**	**W1660**

This bundle is sufficient to perform at least 200 reactions when used at the highest recommended concentration in 96-well plates using a 50µl final reaction volume. Includes:

- 20µl Lumit® Streptavidin-LgBiT
- 20µl Lumit® Streptavidin-SmBiT

Storage Conditions: Store all components at –30°C to –10°C. Before use, mix all components thoroughly. Store all components at –30°C to –10°C immediately after use.

Note: To purchase Lumit® anti-Tag antibodies and strepavidin reagents separately, see Section 7.

3. Before You Begin

General Considerations

Protein interaction buffer: Lumit® Immunoassay Dilution Buffer A, 10X, diluted to 1X concentration with Tris-buffered saline (TBS) or phosphate-buffered saline (PBS) is compatible with many proteins. Some proteins may need a specialized protein interaction buffer with components required for protein stability, activity and binding (e.g., any cofactors, DTT, $MnCl_2$). See Table 1 for additive compatibility.

Preparing and storing reagents: All reagents should be prepared fresh on the day of use. Do not reuse the Lumit® reagent mixtures or the Lumit® detection reagent. We do not recommend storing detection reagent long-term at any temperature.

Plates and instruments: We recommend using round bottom (e.g., Corning® Cat.# 3605) or half-volume (e.g., Corning® Cat.# 3694) white, nonbinding or polypropylene plates. Luminescence can be recorded on a variety of plate readers although the relative light units will depend on the instrument. Assay well geometry and small dispensing volumes may affect mixing efficiency. Thus, poor assay homogeneity in individual wells may result in increased reaction noise, reduced signals or both.

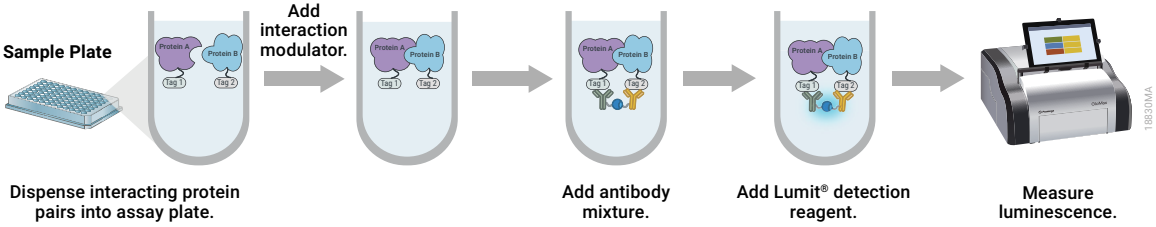

Figure 2. Overview of the Lumit® protein interaction assay add-mix-read protocol.

Table 1. Additive Compatibility with the Lumit® Immunoassay.

Detergent Additives	Compatibility[a]
Triton™ 100	0.002%
Tergitol™	0.002%
Tween® 80	0.01%
Tween® 20	0.02%
Digitonin	0.02%
SDS	0.001%
CHAPS	0.025%
IGEPAL® CA-630	0.005%

Protein Additives	Compatibility[a]
FBS	1.0mg/ml (0.1%)
Serum	
BSA	
Cell lysate	

Other Additives	Compatibility[a]
DMSO	1.0%
Glycerol	
Sucrose	

[a] Additive concentration in the reaction well at which absolute signal will drop by 20%.

4. Guidelines for Developing a Lumit® Protein:Protein Interaction Immunoassay

Protein:protein interactions (PPIs) are fundamental to virtually all processes in a cell and serve as targets for numerous drug therapies. The Lumit® protein interaction immunoassay is a biochemical assay that provides a simple, add-mix-read format for studying PPI and screening for molecules that modulate these interactions (Figure 2).

The protocol below describes how to optimize a Lumit® Immunoassay to detect the interaction between KRAS(G12C)-GppNHp-6HIS and RBD-c-RAF-GST using Lumit® Anti-6His-LgBiT and Anti-6His-SmBiT (Cat.# W1600) and Lumit® Anti-GST-LgBiT and Anti-GST-SmBiT (Cat.# W1620). This protocol serves as an example and can be adapted by substituting KRAS(G12C)-GppNHp-6HIS and RBD-c-RAF-GST with other tagged proteins of interest (indicated as Protein A-Tag 1 and Protein B-Tag 2) and substituting Lumit® Anti-GST and Anti-6His antibodies with Lumit® reagents corresponding to Tag 1 and Tag 2.

Materials to Be Supplied By the User

- two Lumit® anti-Tag antibodies corresponding to Tag 1 and Tag 2 (e.g., Cat.# W1600 and W1620)
- Lumit® Immunoassay Detection Reagent A (Cat.# VB2010) that includes Lumit® Detection Substrate A and Lumit® Immunoassay Dilution Buffer A, 10X
- Protein A-Tag 1 (e.g., KRAS(G12C)-GppNHp-6HIS; BPS Bioscience Cat.# 100641 and/or KRAS(G12C)-GDP-6HIS; BPS Bioscience Cat.# 100640)
- Protein B-Tag 2 (e.g., RBD-c-RAF-GST; BPS Bioscience Cat.# 100519-1)
- Tris-buffered saline (TBS)
- round bottom (e.g., Corning® Cat.# 3605) or half-volume (e.g., Corning® Cat.# 3694) white, nonbinding or polypropylene plates
- luminometer capable of reading multiwell plates (e.g., GloMax® Discover System, Cat.# GM3000)
- reagent reservoir (e.g., Thermo Fisher Scientific Cat.# 8093-11)
- plate shaker
- plate sealer or cover
- **optional:** protein interaction buffer

4.A. Determining Optimal Lumit® Anti-Tag Reagent Concentrations and Orientations

Use the following protocol to test all possible SmBiT and LgBiT combinations of Lumit® anti-Tag 1 and Lumit® anti-Tag 2 reagents at different concentrations. In the top half of the plate, the antibodies are incubated with the interacting pair to provide an assay signal (Figure 3). In the bottom half of the plate, the antibodies are incubated with a negative control to give a background signal (Figure 3). The antibody concentration and SmBiT/LgBiT pair with the highest luminescence and signal-to-background ratio will be used in all subsequent experiments.

Components in each well:

Reagent	Test Condition (top half of plate)	Negative Control (bottom half of plate)
2X protein master mix	20µl	–
2X negative control	–	20µl
4X anti Tag-SmBiT reagent	10µl	10µl
4X anti Tag-LgBiT reagent	10µl	10µl
total volume	**40µl**	**40µl**

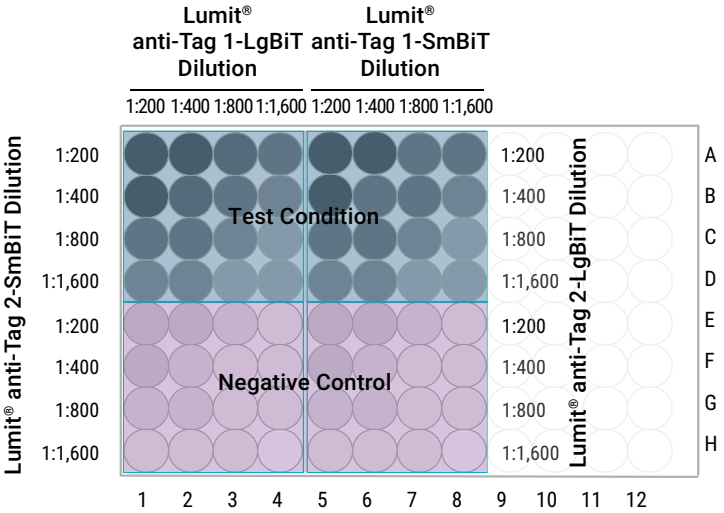

Figure 3. Plate layout for experiment. Dilutions listed are the final dilutions in the well.

4.A. Determining Optimal Anti-Tag Reagent Concentrations and Orientations (continued)

Preparing Protein Master Mix and Negative Control

Choose an appropriate starting concentration based on the affinity of the protein:protein interaction. For protein partners with high-affinity binding (K_d in low nM or less), we recommend concentrations five- to tenfold higher than the K_d. For pairs with lower binding affinity (K_d ≥100nM), the amount of protein required for the assay may become limiting so we recommend a starting concentration of 25–100nM. The K_d of KRAS(G12C)-GppNHp and RBD-c-RAF in the example shown below is ~50nM.

1. Prepare a protein interaction buffer (see Section 3). In this example, a buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM $MgCl_2$ and 1mM DTT was used.
2. Prepare 700µl of a 2X Protein Master Mix containing Protein A-Tag 1 and Protein B-Tag 2 at 2X the desired final concentration in the protein interaction buffer. Here a solution of 200nM KRAS(G12C)-GppNHp-6HIS and 200nM RBD-c-RAF-GST was prepared to provide a 100nM final concentration for both proteins.
3. Prepare 700µl of 2X Negative Control in the protein interaction buffer. For the example assay, buffer alone was used.

   Note: There are several negative control options to consider, including:

   - Protein A-Tag 1 only
   - Protein B-Tag 2 only
   - both interacting partners in presence of an inhibitor
   - Protein A-Tag 1 + Protein B-Tag 2 with an excess of untagged Protein A or untagged Protein B (to compete for binding)
   - buffer alone with no proteins.

   If using a control with protein, the concentration should be identical to what is used in the 2X Protein Master Mix.
4. Incubate to form the protein complex. For this experiment, the proteins were incubated for 60 minutes at room temperature.

Preparing the Anti-Tag Reagent Working Solutions

1. Prepare 5ml of 1X Lumit® dilution buffer by combining 4.5ml of TBS and 500µl of Lumit® Immunoassay Dilution Buffer A, 10X. Use the 1X Lumit® dilution buffer to prepare 4X working solutions described in Step 3 and Lumit® detection reagent in the next section.
2. Remove the anti-Tag reagents from storage, and centrifuge briefly to collect the samples at the bottom of the tube. Remove the desired volume for your experiment and store the remaining anti-Tag antibody at –30°C to –10°C.

3. For each of the four anti-Tag reagents, perform twofold serial dilutions in 1X Lumit® dilution buffer to generate a dilution series of four 4X working solutions at 1:50, 1:100, 1:200 and 1:400 dilutions (Figure 4). Generate 100µl of each dilution.
   a. Dispense 196µl of 1X Lumit® dilution buffer into Tube 1.
   b. Dispense 100µl of 1X Lumit® dilution buffer into Tubes 2–4.
   c. Add 4µl of anti-Tag reagent to Tube 1. Mix thoroughly by pipetting.
   d. Remove 100µl from Tube 1, add to Tube 2 and mix thoroughly by pipetting.
   e. Repeat Step d for Tubes 3 and 4.
   f. Repeat Steps a–e for each of the remaining three anti-Tag reagents.

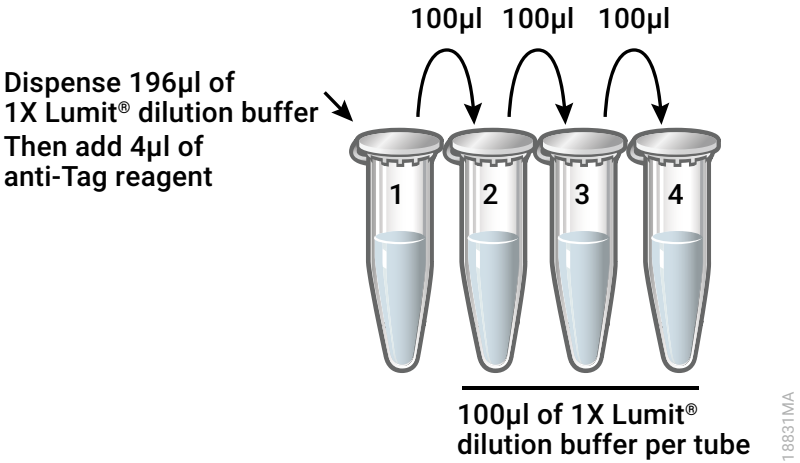

Figure 4. Twofold dilution scheme for the 4X anti-Tag reagent working solutions.

4. Keep 4X working solutions on ice until needed.

Performing the Lumit® Protein:Protein Interaction Immunoassay

1. Add 20µl of 2X Protein Master Mix or 20µl of 2X Negative Control to respective wells, as shown in Figure 3.
2. Add 10µl each of the anti-Tag-SmBiT and anti-Tag-LgBiT antibodies to wells, as shown in Figure 3.
3. Incubate at room temperature with gentle shaking for 30 minutes.
4. Prepare fresh Lumit® detection reagent by mixing 20µl of the Lumit® Detection Substrate A with 980µl of the 1X Lumit® dilution buffer (50-fold dilution).
5. Mix Lumit® detection reagent by inverting the tube, and transfer into a reagent reservoir.
6. Add 10µl of Lumit® detection reagent to each well.
7. Incubate at room temperature with gentle shaking for 3–5 minutes.
8. Measure luminescence using a plate-reading luminometer.

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4.A. Determining Optimal Anti-Tag Reagent Concentrations and Orientations (continued)

Calculations and Analysis

1. Calculate the signal-to-background ratio (Figure 5, Panel B) for each anti-Tag antibody concentration by dividing the interacting pair wells by the corresponding control well (for example, A1 ÷ E1, A2 ÷ E2, A3 ÷ E3, etc.).
2. Select the dilution and SmBiT/LgBiT pair that provides the greatest luminescent signal with the largest signal-to-background ratio to use in future experiments. In this example experiment, a 1:200 dilution of Lumit® Anti-GST-SmBiT and a 1:400 dilution of Lumit® Anti-6His-LgBiT were chosen to use in all subsequent experiments (Figure 5, Panel B).

A.

Lumit® Anti-6His-SmBiT Dilution		Lumit® Anti-GST-LgBiT Dilution 1:200	1:400	1:800	1:1,600	Lumit® Anti-GST-SmBiT Dilution 1:200	1:400	1:800	1:1,600	Lumit® Anti-6His-LgBiT Dilution
KRAS-GppNHp + RBD-c-RAF	1:200	2,467,000	1,145,000	653,500	296,600	2,601,000	1,490,000	728,900	419,600	1:200
	1:400	1,325,000	713,300	372,000	199,300	1,601,000	781,600	412,400	230,700	1:400
	1:800	866,700	422,500	202,000	107,700	815,500	453,300	234,900	131,300	1:800
	1:1,600	458,300	222,200	115,300	53,720	418,700	228,600	105,400	59,920	1:1,600
Buffer Only	1:200	180,700	86,270	41,390	26,160	124,800	70,210	49,160	32,000	1:200
	1:400	122,500	64,300	36,330	18,610	67,350	42,270	26,530	16,570	1:400
	1:800	87,130	39,350	21,500	11,320	47,520	26,110	14,840	10,790	1:800
	1:1,600	55,670	28,140	16,300	8,353	29,640	16,750	9,424	6,782	1:1,600

B.

Lumit® Anti-6His-SmBiT Signal-to-Background Ratio	Lumit® Anti-GST-LgBiT Signal-to-Background Ratio 1:200	1:400	1:800	1:1,600	Lumit® Anti-GST-SmBiT Signal-to-Background Ratio 1:200	1:400	1:800	1:1,600	Lumit® Anti-6His-LgBiT Signal-to-Background Ratio
1:200	14	13	16	11	21	21	15	13	1:200
1:400	11	11	10	11	24	18	16	14	1:400
1:800	10	11	9	10	17	17	16	12	1:800
1:1,600	8	8	7	6	14	14	11	9	1:1,600

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Figure 5. Determining the best Anti-Tag 1 and Anti-Tag 2 reagent combination and dilution. Panel A. Relative luminescence units (RLUs) from the Figure 3 plate layout experiment with KRAS(G12C)-GppNHp-6HIS and RBD-c-RAF-GST. **Panel B.** Signal-to-background ratio calculated from Panel A. Based on the data, a 1:200 dilution of Lumit® Anti-GST-SmBiT and a 1:400 dilution Lumit® Anti-6His-LgBiT were selected for subsequent experiments (in blue cell).

4.B. Determining the Optimal Concentrations for Proteins of Interest

The goal for this experiment is to determine the optimal concentrations for the proteins of interest using the anti-Tag reagent concentrations chosen in Section 4.A. Consider choosing the lowest protein concentrations that give an acceptable signal-to-background ratio. In this example experiment, KRAS(G12C)-GppNHp-6HIS and RBD-c-RAF-GST were titrated starting at 200nM final using the anti-Tag antibody combination and concentrations identified in Section 4.A.

Components in each well:

Reagent	Volume
4X Protein A-Tag 1	10µl
4X Protein B-Tag 2	10µl
2X anti-Tag antibody master mix	20µl
total volume	**40µl**

Preparing Protein Dilutions

1. Prepare a protein interaction buffer (see Section 3). For this assay, a buffer containing 50mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM $MgCl_2$ and 1mM DTT was used. You will need 2ml of buffer.
2. For each protein, prepare seven twofold dilutions to generate a 4X working concentration. An eighth tube contains only buffer and serves as the background for the signal-to-background calculation (Figure 6). Generate 100µl of each dilution.
   a. Dispense 200µl of protein interaction buffer into Tube 1.
   b. Dispense 100µl of protein interaction buffer into Tubes 2–8.
   c. Add Protein A-Tag 1 to Tube 1 at desired concentration to generate a 4X working solution. Mix thoroughly by pipetting. In this example, the starting concentration was 800nM KRAS(G12C)-GppNHp-6HIS (Tube 1).
   d. Remove 100µl from Tube 1, add to Tube 2 and mix thoroughly by pipetting.
   e. Repeat Step d for Tubes 3–7.

   Note: The background (buffer only) wells are important controls and need to be included.

4.B. Determining the Optimal Concentrations for Proteins of Interest (continued)

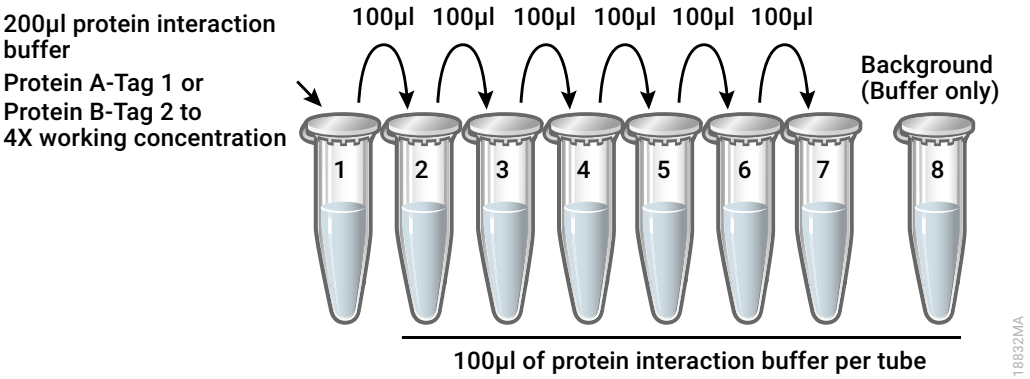


Figure 6. Twofold dilution scheme for the 4X protein working concentrations.

3. Repeat Step 2 with Protein B-Tag 2. In this assay, RBD-c-RAF was used at the same concentrations as KRAS(G12C)-GppNHp-6HIS, as described in Step 2.

Preparing the Anti-Tag Reagent Master Mix

1. Prepare 5ml of 1X Lumit® dilution buffer by combining 4.5ml of TBS and 500µl of Lumit® Immunoassay Dilution Buffer A, 10X. The 1X Lumit® dilution buffer is used to prepare the 2X antibody master mix described in Step 2 and the detection reagent in the next section.
2. Prepare 1.5ml of 2X anti-Tag antibody master mix in 1X Lumit® dilution buffer containing the anti-Tag-SmBiT and anti-Tag-LgBiT at 2X the concentrations determined in Section 4.A.
3. Keep on ice until use.

Performing the Lumit® Protein:Protein Interaction Immunoassay

1. Add 10µl of Protein A-Tag 1 and 10µl of Protein B-Tag 2 prepared above to the wells as shown in Figure 7, Panel A.
2. Incubate to form the protein complex. For this experiment, the proteins were incubated for 60 minutes at room temperature.
3. Add 20µl of 2X anti-Tag antibody master mix to each well.
4. Incubate at room temperature for 30 minutes.
5. Prepare Lumit® detection reagent by adding 20µl of Lumit® Detection Substrate A to 1ml of 1X Lumit® dilution buffer.
6. Mix Lumit® detection reagent by inverting the tube, and transfer into a reagent reservoir.
7. Add 10µl of Lumit® detection reagent to the wells.
8. Incubate the plate at room temperature with gentle shaking for 3–5 minutes.
9. Measure luminescence using a plate-reading luminometer.

Calculations and Analysis

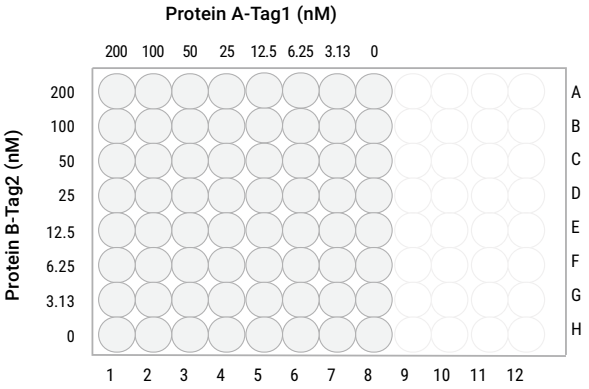
1. Select the protein concentrations that give the highest luminescence signal with the best signal-to-background ratio to use in future experiments. In this example experiment, concentrations greater than 100nM RBD-c-RAF-GST show decreasing luminescent measurements, indicating that the anti-GST antibody is saturated (Figure 7, Panel B). However, KRAS(G12C)-GppNHp does not reach a similar point of decreasing luminscence, indicating that, if desired, the protein concentration can be increased to potentially increase the signal-to-background ratio.

   Note: In some cases, increasing protein concentration no longer increases the luminescence indicating that the antibody is saturated. Choose protein concentrations below this saturated level.

2. Select the best concentrations to use in subsequent assays. In this example, while 100nM RBD-c-RAF and 200nM KRAS(G12C)-GppNHp showed the best signal-to-background results, 50nM of each protein was chosen for subsequent assays because this concentration provides an acceptable signal-to-background ratio for our downstream applications (Figure 7, Panel C).

4.B. Determining the Optimal Concentrations for Proteins of Interest (continued)

A.



B.

RDB-cRAF (nM)

KRAS(G12C)-GppNHp (nM)	200	100	50	25	12.5	6.25	3.13	0
200	1,933,000	2,406,000	2,310,000	1,757,000	841,400	462,400	260,200	46,980
100	1,488,000	1,625,000	727,000	500,200	663,300	327,500	247,400	50,820
50	962,800	1,025,000	989,200	745,900	610,900	418,000	249,900	57,840
25	529,100	601,700	579,300	567,000	548,900	421,100	267,000	62,420
12.5	286,100	333,200	389,100	370,400	457,000	335,500	250,400	63,320
6.25	157,900	190,900	248,400	269,800	312,400	246,900	184,100	66,660
3.13	114,200	127,000	164,400	223,100	234,100	193,900	151,900	70,480
0	83,540	94,120	108,100	115,700	109,700	94,170	88,710	64,200

C.

RDB-cRAF Signal-to-Background Ratio

KRAS(G12C)-GppNHp Signal-to-Background Ratio	200	100	50	25	12.5	6.25	3.13	0
200	30.1	37.5	36.0	27.4	13.1	7.2	4.1	0.7
100	23.2	25.3	11.3	7.8	10.3	5.1	3.9	0.8
50	15.0	16.0	15.4	11.6	9.5	6.5	3.9	0.9
25	8.2	9.4	9.0	8.8	8.5	6.6	4.2	1.0
12.5	4.5	5.2	6.1	5.8	7.1	5.2	3.9	1.1
6.25	2.5	3.0	3.9	4.2	4.9	3.8	2.9	1.0
3.13	1.8	2.0	2.6	3.5	3.6	3.0	2.4	1.1
0	1.3	1.5	1.7	1.8	1.7	1.5	1.4	1.0

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Figure 7. Determining the optimal KRAS(G12C)-GppNHp-6HIS and RBD-c-RAF-GST concentration. Panel A. The experiment was set up using a dilution series of KRAS(G12C)-GppNHp-HIS and c-RAF-GST with a 1:200 dilution of Lumit® Anti-GST-SmBiT and a 1:400 dilution of Lumit® Anti-6His-LgBiT. Concentrations indicated are the final concentrations in the well. **Panel B.** Relative luminescence units (RLUs) from the experiment in Panel A. **Panel C.** Signal-to-background ratio calculated from Panel B with 50nM of each protein selected for subsequent experiments (in blue cell).

4.C. Protein/Inhibitor Experiment

One possible downstream application after establishing Lumit® PPI experimental parameters in Sections 4.A and 4.B is to modulate the interaction with small molecule inhibitors. For any downstream experiments, additional Lumit® anti-Tag reagents may be needed and can be purchased separately. The following protocol is one way to set up an experiment with protein inhibitors, but the format is flexible and may be modified as desired.

In this example experiment, the concentrations established in Sections 4.A and 4.B were used to monitor the effect of KRAS-inhibitor AMG510 on the interaction between KRAS(G12C)-GDP-6HIS and RBD-c-RAF-GST.

Components in each well:

Reagent	Volume
8X Protein A-Tag 1	5µl
8X Protein B-Tag 2	5µl
4X PPI inhibitor dilution series	10µl
2X anti-Tag antibody master mix	20µl
total volume	**40µl**

Preparing Protein Master Mix and PPI Inhibitor Dilution Series

1. Prepare a protein interaction buffer. The protein interaction buffer contains 50mM Tris-HCl (pH 7.5), 150mM NaCl and 1mM DTT (See Section 3.) In this example assay, a second buffer, nucleotide exchange buffer, is required for KRAS(G12C)-GDP-6HIS to promote GDP to GTP exchange. The nucleotide exchange buffer contains 50mM Tris-HCl (pH 7.5), 150mM NaCl, 5mM EDTA, 0.5mM $MgCl_2$, 1mM DTT and 10µM GTP.
2. Prepare proteins by either:
   a. Making a 4X protein master mix by adding Protein A-Tag 1 and Protein B-Tag 2 at 4X the concentrations chosen in Section 4.B **or**
   b. Separately diluting Protein A-Tag 1 and Protein B-Tag 2 to 8X concentration chosen in Section 4.B.

   In this experiment, KRAS(G12C)-GDP-6HIS was diluted to 400nM in the nucleotide exchange buffer and RBD-c-RAF-GST diluted to 400nM in the protein interaction buffer.
3. Generate a dilution series of the selected inhibitor at a 4X working concentration, ensuring one well is buffer only as a negative control. For this assay, seven threefold dilutions starting at 10µM were prepared in nucleotide exchange buffer.

4.C. Protein/Inhibitor Experiment (continued)

Preparing the Anti-Tag Reagent Master Mix

1. Prepare 5ml of 1X Lumit® dilution buffer by combining 4.5ml of TBS and 500µl of Lumit® Immunoassay Dilution Buffer A, 10X. The 1X Lumit® dilution buffer is used to make the 2X master mix described in Step 2 and the detection reagent in the next section.
2. Make a 2X anti-Tag antibody master mix in 1X Lumit® dilution buffer containing the anti-Tag-SmBiT and anti-Tag-LgBiT at 2X the concentrations determined in Section 4.A. In this assay, a 1:200 dilution of Lumit® Anti-GST-SmBiT and 1:400 dilution of Lumit® Anti-6His-LgBiT were used.
3. Store on ice until use.

Performing the Lumit® Protein:Protein Interaction Immunoassay

1. Add proteins and 4X PPI inhibitor dilution to wells.
   a. If a 4X protein master mix is used, add 10µl of the 4X protein master mix and 10µl of 4X PPI inhibitor dilution to wells.
   b. If the proteins were diluted separately and you want to preincubate inhibitor with one of the proteins, incubate 5µl of 8X Protein A-Tag 1 and 10µl of 4X inhibitor dilution series together before adding 5µl of 8X Protein B-Tag 2.

   In this assay example, 5µl of KRAS(G12C)GDP-6HIS and 10µl of inhibitor dilution series were mixed. After a 10-minute incubation, 10mM $MgCl_2$ was dispensed to each well to stabilize the newly formed KRAS(G12C)-GTP complex before adding 5µl of c-RAF-GTP.
2. Incubate to form the protein complex. In this example, the mixture was incubated for 30 minutes.
3. Add 20µl of 2X anti-Tag antibody master mix to each well.
4. Incubate at room temperature for 30 minutes.
5. Mix the previously prepared Lumit® detection reagent by inverting the tube, and transfer into a reagent reservoir.
6. Add 10µl of Lumit® detection reagent to the wells.
7. Incubate the plate at room temperature for 3–5 minutes.
8. Measure luminescence using a plate-reading luminometer.

A.

AMG510 (µM)	Luminescence (RLU)		
10.0	107,600	123,800	152,000
3.33	221,100	252,600	249,500
1.11	615,800	644,600	624,400
0.370	904,600	1,027,000	972,000
0.123	1,034,000	1,102,000	1,044,000
0.041	1,093,000	1,070,000	1,057,000
0.014	1,066,000	1,070,000	1,058,000
0	1,164,000	1,203,000	1,133,000

B.

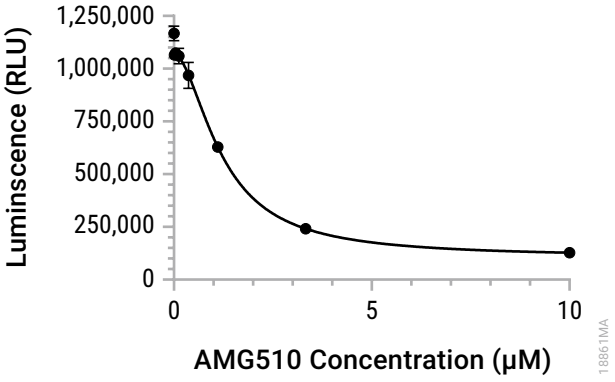

Figure 8. Inhibiting the KRAS-c-RAF interaction with AMG510. Panel A. Luminescence with different concentrations of AMG510. **Panel B.** Inhibition graph fitted to a 4P nonlinear regression. IC_{50} = 1.2µM.

5. Guidelines for Developing a Lumit® Protein:Small Molecule Immunoassay

The binding of a small molecule to a target protein is the basis of many drugs, and can function by inhibiting enzymatic activity, interfering with protein interactions, or bringing two proteins together. The Lumit® protein:small molecule immunoassay is a simple biochemical assay for screening of protein:small molecule interactions.

This protocol describes how to optimize a Lumit® Immunoassay to detect the interaction between His-tagged Bruton's tyrosine kinase (BTK) and a biotinylated small-molecule drug, ibrutinib, using Lumit® Anti-6His-LgBiT and Anti-6His-SmBiT (Cat.# W1600) and Lumit® Streptavidin-LgBiT and Streptavidin-SmBiT (Cat.# W1660). This protocol provides an example experiment and can be adapted by substituting other tagged proteins and/or biotinylated small molecules.

Materials to Be Supplied by the User

- Lumit® anti-Tag antibodies corresponding to Tag 1 (e.g., Cat.# W1600)
- Lumit® Strepavidin-LgBiT and Lumit® Strepavidin-SmBiT (Cat.# W1660)
- Lumit® Immunoassay Detection Reagent A (Cat.# VB2010) that includes Lumit® Detection Substrate A and Lumit® Immunoassay Dilution Buffer A, 10X
- Protein A-Tag 1 (e.g., BTK-His tag Kinase Enzyme System [Cat.# V2941])
- biotinylated small molecule (e.g., Ibrutinib-Biotin MedChemExpress; Catalog# HY-100342)
- TBS
- round bottom (e.g., Corning® Cat.# 3605) or half-volume (e.g., Corning® Cat.# 3694) white, nonbinding or polypropylene plates
- luminometer capable of reading multi-well plates (e.g., GloMax® Discover System, Cat.# GM3000)
- reagent reservoir (e.g., Thermo Fisher Scientific Cat.# 8093-11)
- plate shaker
- plate sealer or cover
- **optional:** protein interaction buffer

5.A. Determining the Optimal Lumit® Reagent Concentrations and Orientations

Use the following protocol to test different concentrations of anti-Tag 1-LgBiT + Lumit® Streptavidin-SmBiT and anti-Tag 1-SmBiT + Lumit® Streptavidin-LgBiT. In the top half of the plate, the Lumit® reagents are incubated with the protein-small molecule pair to measure the assay signal. In the bottom half of the plate, the Lumit® reagents are incubated with a negative control to measure the background signal. The Lumit® reagent concentrations and SmBiT/LgBiT pair with the highest luminescence and signal-to-background ratio will be used in all subsequent experiments.

Components in each well:

Reagent	Test Condition (top half of plate)	Negative Control (bottom half of plate)
2X protein-small molecule master mix	20µl	–
2X negative control	–	20µl
4X Anti Tag-SmBiT antibody	10µl	10µl
4X Anti Tag-LgBiT antibody	10µl	10µl
total volume	**40µl**	**40µl**

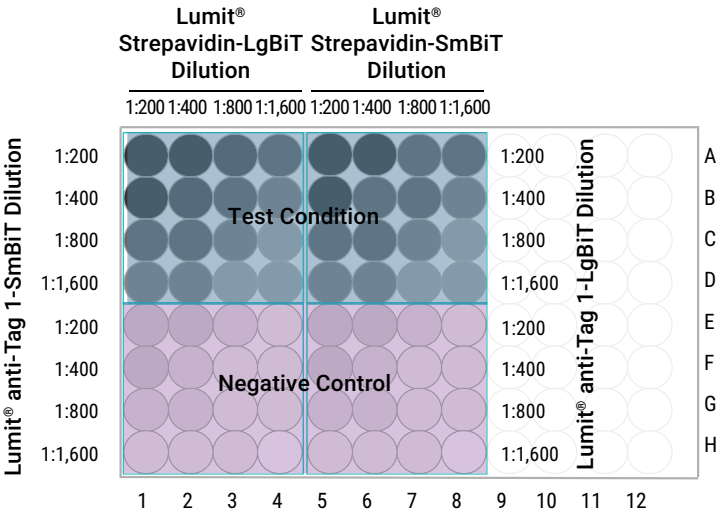

Figure 9. Plate layout for experiment. Dilutions indicated are the final dilutions in the well.

5.A. Determining the Optimal Lumit® Reagent Concentrations and Orientations (continued)

Preparing the Protein-Small Molecule Mix and Negative Control

Choose an appropriate starting concentration based on the affinity between the protein and small molecule. If possible, we recommend protein concentrations two- to fivefold higher than the K_d for high-affinity binding (K_d in low nanomolar or less). For pairs with lower binding affinity (K_d≥100nM), the amount of protein required for the assay may become limiting, so we recommend a starting concentration of 50–250nM. IC_{50} for the BTK-Ibrutinib interaction shown below is ~1.0nM.

1. Prepare a protein interaction buffer. (See Section 3.) For this example, Reaction Buffer provided in BTK-His tag Kinase Enzyme System (Cat.# V2941) was used.
2. Prepare 700µl of a 2X protein-small molecule master mix containing Protein A-Tag 1 and a small molecule fused with biotin at 2X of your desired final concentration. Here a solution of 30nM BTK-HIS and 30nM biotinylated ibrutinib was prepared, providing a 15nM final concentration for each component.
3. Prepare 700µl of negative control master mix. Here we used buffer alone.

   Note: There are several negative control options to consider, including:

   - Protein A-Tag 1 only;
   - small molecule only;
   - both interacting partners with an excess of untagged interacting partner to compete for binding;
   - buffer alone. If using a control with protein or small molecule, the concentration should be identical to what is used in the 2X protein-small molecule master mix.
4. Incubate to form the protein-small molecule complex. For this experiment, the protein and small molecule were incubated for 60 minutes at room temperature.

Preparing the Lumit® Reagent Master Mix

1. Prepare 5ml of 1X Lumit® dilution buffer by combining 4.5ml of TBS and 500µl of Lumit® Immunoassay Dilution Buffer A, 10X. Use the 1X Lumit® dilution buffer to prepare 4X working solutions described in Step 3 and Lumit® detection reagent in the next section.
2. Remove the Lumit® reagents from storage, and centrifuge briefly to collect the samples at the bottom of the tube. Remove the desired volume for your experiment and store the remaining reagents at –30°C to –10°C.
3. For each Lumit® reagent, perform twofold serial dilutions in 1X Lumit® dilution buffer to generate four 4X working solutions at 1:50, 1:100, 1:200 and 1:400 dilutions (Figure 10). You will need 100µl of each dilution.
   a. Dispense 196µl of 1X Lumit® dilution buffer into Tube 1.
   b. Dispense 100µl of 1X Lumit® dilution buffer into Tubes 2–4.
   c. Add 4µl of Lumit® reagent to Tube 1. Mix thoroughly by pipetting.
   d. Remove 100µl from Tube 1, add to Tube 2 and mix thoroughly by pipetting.
   e. Repeat Step d for Tubes 3 and 4.
   f. Repeat Steps a–e for each of the three remaining Lumit® reagents.

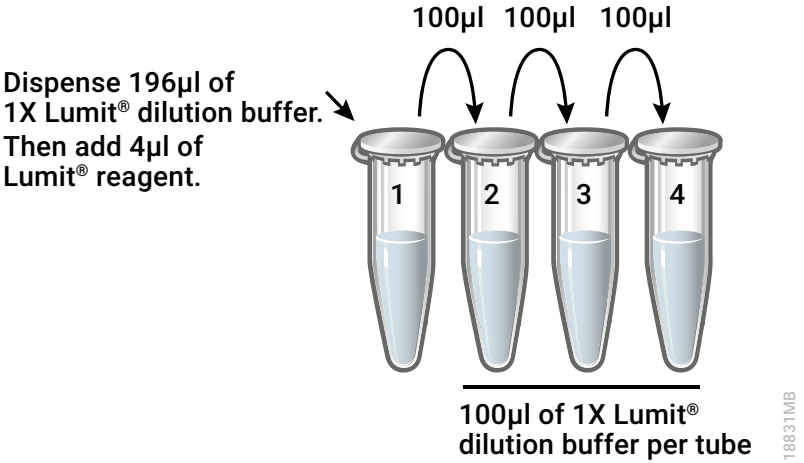

Figure 10. Twofold dilution scheme for the 4X Lumit® reagent working solutions.

4. Keep 4X working solutions on ice until needed.

Performing the Lumit® Protein:Small Molecule Interaction Immunoassay

1. Add 20µl of 2X protein-small molecule master mix or 20µl of 2X negative control to respective wells shown in Figure 9.
2. Add 10µl each of the anti-Tag-SmBiT and anti-Tag-LgBiT to wells, as shown in Figure 9.
3. Incubate at room temperature with gentle shaking for 30 minutes.
4. Prepare Lumit® detection reagent by adding 20µl of Lumit® Detection Substrate A to 1ml of 1X Lumit® dilution buffer.
5. Mix the Lumit® detection reagent by inverting the tube, and transfer into a reagent reservoir.
6. Add 10µl of Lumit® detection reagent to the wells.
7. Incubate at room temperature with gentle shaking for 3–5 minutes.
8. Measure luminescence using a plate-reading luminometer.

5.A. Determining the Optimal Lumit® Reagent Concentrations and Orientations (continued)

Calculations and Analysis

1. Calculate the signal-to-background ratio (Figure 11, Panel B) for each Lumit® reagent concentration by dividing the wells with the interacting pair by the corresponding control well (for example, A1 ÷ E1, A2 ÷ E2, A3 ÷ E3, etc).
2. Select the concentration and SmBiT/LgBiT pair that provides the greatest luminescent signal with the largest signal-to-background ratio to use in future experiments. In this example experiment, the 1:200 dilution of Lumit® Anti-6His-LgBiT and 1:400 dilution of Lumit® Streptavidin-SmBiT were chosen to use in all subsequent experiments (Figure 11, Panel B).

A.

Lumit® Anti-6His-SmBiT Dilution		Lumit® Strepavidin-LgBiT Dilution 1:200	1:400	1:800	1:1,600	Lumit® Strepavidin-SmBiT Dilution 1:200	1:400	1:800	1:1,600	Lumit® Anti-6His-LgBiT Dilution
BTK + Ibrutinib	1:200	10,360,000	10,210,000	1,875,000	364,000	15,540,000	9,064,000	1,219,000	457,000	1:200
	1:400	5,448,000	5,270,300	1,088,000	176,400	8,002,000	4,710,000	1,048,000	187,100	1:400
	1:800	2,795,000	3,011,000	771,200	112,600	3,966,000	2,545,000	545,600	103,300	1:800
	1:1,600	1,601,000	1,666,000	386,900	84,370	1,948,000	1,287,000	321,500	66,060	1:1,600
Buffer Only	1:200	557,100	294,500	166,200	100,900	406,900	229,900	144,800	101,400	1:200
	1:400	371,400	201,600	115,100	72,890	245,100	135,900	87,790	64,150	1:400
	1:800	274,200	152,700	88,140	67,770	154,800	89,770	60,650	45,120	1:800
	1:1,600	229,400	132,800	81,410	54,810	114,400	70,920	49,390	37,370	1:1,600

B.

Lumit® Anti-6His-SmBiT Signal-to-Background Ratio	Lumit® Strepavidin-LgBiT Signal-to-Background Ratio 1:200	1:400	1:800	1:1,600	Lumit® Strepavidin-SmBiT Signal-to-Background Ratio 1:200	1:400	1:800	1:1,600	Lumit® Anti-6His-LgBiT Signal-to-Background Ratio
1:200	19	35	11	4	38	39	8	5	1:200
1:400	15	26	9	2	33	35	12	3	1:400
1:800	10	20	9	2	26	28	9	2	1:800
1:1,600	7	13	5	2	17	18	7	2	1:1,600

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Figure 11. Determining the best Anti-Tag 1 antibody and Lumit® Streptavidin combination and dilution. Panel A. Relative luminescence units (RLUs) from the Figure 9 plate layout experiment with BTK-His and biotinylated ibrutinib. **Panel B.** Signal-to-background ratio calculated from Panel A. Based on the data, a 1:200 dilution of Lumit® Anti-6His-LgBiT and 1:400 dilution of Lumit® Streptavidin-SmBiT were selected to use in all subsequent experiments (blue cell).

5.B. Selecting the Optimal Concentration for the Protein of Interest and Biotinylated Small Molecule

The goal for the next experiment is to determine the optimal concentrations for the protein of interest and biotinylated small molecule using the reagent concentrations selected in Section 5.A. We recommend choosing the lowest protein concentration that gives an acceptable signal-to-background ratio. Try a broad range of small molecule titrations, but for the chosen protein you may want to do one of the following:

1. titrate the protein (see Section 4.B),
2. choose a few protein concentrations, or
3. use the protein concentration from Section 5.A.

Here, ibrutinib-biotin was titrated starting at 300nM with a few different BTK-His concentrations.

Components in each well:

Reagent	Volume
4X Protein A-Tag 1	10µl
4X biotinylated small molecule	10µl
2X anti-Tag antibody master mix	20µl
total	**40µl**

Preparing Samples

1. Prepare a protein interaction buffer. (See Section 3.) In this experiment, the Reaction Buffer provided with the BTK Kinase Enzyme System (Cat.# V2941) was used.
2. If desired, prepare a dilution series of Protein A-Tag 1 at a 4X working concentration in your interaction buffer. Here, BTK-His was diluted to 60nM, 30nM and 15nM for final concentrations of 15nM, 7.5nM and 3.75nM.
3. Prepare seven twofold dilutions of the biotinylated small molecule to generate 4X working concentration. An eighth tube contains only buffer and serves as the background for the signal-to-background calculation (Figure 12). Make 100µl of each concentration.
    a. Dispense 200µl of protein interaction buffer into Tube 1.
    b. Dispense 100µl of protein interaction buffer into Tubes 2–8.
    c. Add the biotinylated small molecule to Tube 1 at desired concentration to generate a 4X working solution. Mix thoroughly by pipetting. In this example, the starting concentration was 1.2µM ibrutinib-biotin (Tube 1).
    d. Remove 100µl from Tube 1, add to Tube 2 and mix thoroughly by pipetting.
    e. Repeat Step d for Tubes 3–7.

    Note: In this example, the final concentrations range from 300nM to 4.7nM.

5.B. Selecting the Optimal Concentration for the Protein of Interest and Biotinylated Small Molecule (continued)

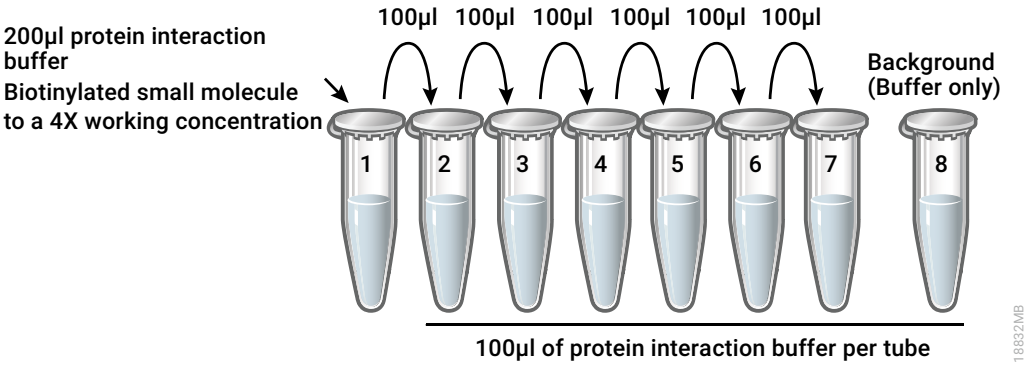

Figure 12. Twofold dilution scheme for the 4X biotinylated small molecule working concentrations.

Preparing the Anti-Tag Reagent Master Mix

1. Prepare 5ml of 1X Lumit® dilution buffer by combining 4.5ml of TBS and 500µl of Lumit® Immunoassay Dilution Buffer A, 10X. Use the 1X Lumit® dilution buffer to prepare 2X working solutions described in Step 2 and Lumit® detection reagent in the next section.
2. Prepare a 2X anti-Tag reagent master mix in 1X Lumit® dilution buffer containing a 2X concentration of the anti-Tag reagents determined in Section 5.A.
3. Store on ice until use.

Performing the Lumit® Protein:Small Molecule Interaction Immunoassay

1. Add 10µl of Protein A-Tag 1 and 10µl of biotinylated small molecule prepared above to the wells as shown in Figure 13, Panel A.
2. Incubate at room temperature for 30–60 minutes with gentle shaking.
3. Add 20µl of anti-Tag reagent master mix to each well.
4. Incubate at room temperature for 30 minutes.
5. Prepare fresh Lumit® detection reagent by mixing 20µl of the Lumit® Detection Substrate A with 980µl of the 1X Lumit® dilution buffer (50-fold dilution).
6. Mix Lumit® detection reagent by inverting the tube, and transfer into reagent reservoir.
7. Add 10µl of Lumit® detection reagent to the wells.
8. Incubate the plate at room temperature with gentle shaking for 3–5 minutes.
9. Measure luminescence using a plate-reading luminometer.

Calculations and Analysis

1. Select the concentrations that provide the highest luminescence signal with the best signal-to-background ratio to use in future experiments. In this example, concentrations greater than 75nM ibrutinib-biotin show decreasing luminescent measurements, indicating that the biotinylated small molecule is saturated. However, the chosen BTK-His concentrations do not reach a similar point of decreasing luminscence (Figure 13, Panel A). This means that at 75nM ibrutinib-biotin, streptavidin was saturated, and to saturate anti-6His, the BTK-His concentration needs to be greater than 15nM.
2. Select the best concentrations for subsequent assays. In this example, 15nM BTK-His and 37.5nM ibrutinib-biotin were chosen (Figure 13, Panel B).

A.

		Ibrutinib-Biotin (nM)							
		300	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.6875**	**0**
BTK-His (nM)	**15**	548,900	3,709,000	6,829,000	6,658,000	5,084,000	2,856,000	1,373,000	118,900
	7.5	707,000	2,236,000	3,210,000	2,967,000	2,366,000	1,587,000	758,000	109,700
	3.75	343,300	1,181,000	1,986,000	1,786,000	1,558,000	896,100	604,200	133,100

B.

		Ibrutinib-Biotin (nM)							
		300	**150**	**75**	**37.5**	**18.75**	**9.375**	**4.6875**	**0**
BTK-His (nM)	**15**	5	31	57	56	43	24	12	1
	7.5	6	20	29	27	22	14	7	1
	3.75	3	9	15	13	12	7	5	1

Figure 13. Determining the optimal concentration of BTK-His and ibrutinib-biotin. Panel A. The experiment was set up using a dilution series of BTK-His and ibrutinib-biotin. **Panel B.** Signal-to-background ratios calculated from Panel A resulted in selecting 37.5nM ibrutinib and 15nM BTK for subsequent experiments (in blue cell).

5.C. Inhibitor Competition Experiments

After establishing the Lumit® Protein:Small Molecule Interaction Immunoassay experimental parameters in Sections 5.A and 5.B, one possible downstream application is to test the relative potency of other protein inhibitors in a competitive assay. In this experiment, the biotinylated small molecule is the tracer and the protein is the target. Unlabeled inhibitors are titrated, which will compete with the tracer binding, causing a decrease in signal. For these and other downstream experiments, additional Lumit® anti-Tag reagents may be needed and can be purchased separately (see Section 7). The following protocol is an example competition assay using biotinylated ibrutinib, BTK-His and unlabeled ibrutinib.

Note: While the following experiment can determine the relative IC_{50} values and rank drug potency, calculating actual IC_{50} values requires a number of parameters to be met, including a K_d tenfold higher than the concentration of the biotinylated small molecule, and a tracer concentration that is tenfold higher than the protein target concentration.

Components in each well:

Reagent	Volume
4X tracer + inhibitor master mix	10µl
4X Protein A-Tag 1	10µl
2X anti-Tag reagent master mix	20µl
total volume	**40µl**

Preparing Samples

1. Prepare a protein interaction buffer. (See Section 3.) In this experiment, the Reaction Buffer provided with the BTK Kinase Enzyme System (Cat.# V2941) was used.
2. Dilute tracer in the protein interaction buffer to 4X your desired final concentration. In this experiment, 150nM ibrutinib-biotin was added to provide a 37.5nM final concentration.
3. Using the buffer containing tracer, prepare an inhibitor dilution series at a 4X working concentration, adding buffer containing tracer only to the final tube (similar to the dilution series depicted in Figure 12). For this experiment, the first tube of the dilution series contained 4µM unlabeled ibrutinib, which yielded a 1µM final concentration.
4. Dilute Protein A-Tag 1 to a 4X working concentration in the protein interaction buffer. In this example experiment, 60nM BTK-His was added.

Preparing the Anti-Tag Reagent Master Mix

1. Prepare 5ml of 1X Lumit® dilution buffer by combining 4.5ml of TBS and 500µl of Lumit® Immunoassay Dilution Buffer A, 10X. Use the 1X Lumit® dilution buffer to prepare the 2X master mix described in Step 2 and the detection reagent in the next section.
2. Prepare 1.5ml of 2X anti-Tag reagent master mix in 1X Lumit® dilution buffer containing the anti-Tag-SmBiT and anti-Tag-LgBiT reagents at 2X the concentrations determined in Section 5.A.
3. Store on ice until use.

Performing the Lumit® Protein:Small Molecule Interaction Immunoassay

1. Add 10µl of Protein A-Tag 1 to each well.
2. Dispense 10ul of the serially diluted 4X tracer + inhibitor master mix prepared above to the wells as indicated in Figure 14, Panel A. Each dilution was run in triplicate.
3. Incubate to form the protein complex. For this experiment, the protein, tracer and inhibitor were incubated for 60 minutes at room temperature.
4. Add 20µl of 2X anti-Tag reagent master mix to each well.
5. Incubate at room temperature for 30 minutes.
6. Prepare Lumit® detection reagent by adding 20µl of Lumit® Detection Substrate A to 1ml of 1X Lumit® dilution buffer.
7. Mix Lumit® detection reagent by inverting the tube, and transfer into a reagent reservoir.
8. Add 10µl of Lumit® detection reagent to the wells.
9. Incubate the plate at room temperature for 3–5 minutes.
10. Measure luminescence using a plate-reading luminometer.

A.

	Ibrutinib (nM)									
1,000	**500**	**250**	**125**	**62.5**	**31.3**	**15.6**	**7.81**	**3.91**	**1.95**	**0**
145,200	158,400	169,300	233,300	330,800	695,400	1,126,000	2,423,000	3,171,000	4,134,000	3,632,000
174,300	172,800	189,900	288,300	391,400	859,600	1,397,000	3,012,000	3,805,000	4,383,000	4,884,000
180,800	173,200	206,100	241,400	380,200	745,600	1,582,000	2,459,000	3,497,000	3,943,000	4,230,000

B.

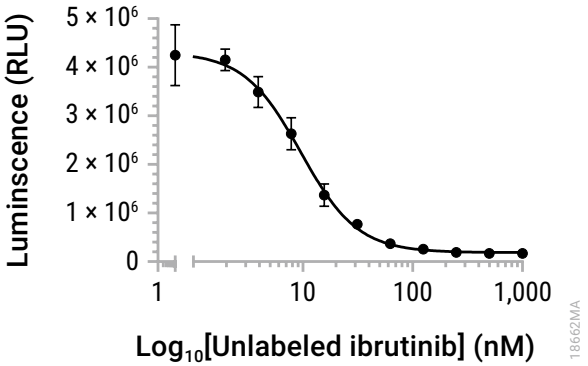

Figure 14. Competition experiment using unlabeled ibrutinib. Panel A. Luminescence measured for an unlabeled ibrutinib titration. **Panel B.** Inhibitor competition graph fitted to a 4P nonlinear regression. The IC_{50} is 8nM.

6. Troubleshooting

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

Symptoms	Causes and Comments
Low signal	Not enough protein. Because low-affinity interactions have a smaller "bound" fraction, more protein is required to detect the interaction. Try using concentrations greater than the K_d.
	Missing buffer components. Although simple buffers like TBS work in some cases, some protein:protein interactions require reducing agents, metals or specific buffering agents. Ensure your proteins are in an appropriate buffer.
	Not enough anti-Tag reagents. The anti-Tag reagent concentrations that give the best signal to background depend upon the protein concentration used (e.g., using 100nM of two proteins may require a 1:200 dilution of each anti-Tag reagent, while 1nM will need less). If protein concentration is increased after determining the initial antibody concentration, reoptimize by running the anti-Tag reagent experiment described in Section 4.A or 5.A.
High background	Too much anti-Tag reagent. Although higher anti-Tag reagent concentrations increase total signal, it also increases background signal. Ensure the anti-Tag reagent concentration determined in Section 4.A or 5.A is appropriate.
	Adjust the buffer. The Lumit® Immunoassay Dilution Buffer A, 10X, has a blocking agent to decrease background signal. Consider using this buffer for the protein interaction buffer in experiments. Add any necessary cofactors or salts to maintain protein stability and function.

7. Related Products

Product	Size	Cat.#
Lumit® Anti-6His-LgBiT	200µl	W1601
Lumit® Anti-6His-SmBiT	200µl	W1611
Lumit® Anti-GST-LgBiT	200µl	W1621
Lumit® Anti-GST-SmBiT	200µl	W1631
Lumit® Anti-DYKDDDDK-LgBiT	200µl	W1641
Lumit® Anti-DYKDDDDK-SmBiT	200µl	W1651
Lumit® Streptavidin-LgBiT	200µl	W1661
Lumit® Streptavidin-SmBiT	200µl	W1671
Lumit® Immunoassay Detection Reagent A	500 assays	VB2010
	5,000 assays	VB2020
	50,000 assays	VB2030
Lumit® Anti-Mouse Ab-LgBiT	30µl	W1021
	300µl	W1022
Lumit® Anti-Mouse Ab-SmBiT	30µl	W1051
	300µl	W1052
Lumit® Anti-Rabbit Ab-LgBiT	30µl	W1041
	300µl	W1042
Lumit® Anti-Rabbit Ab-SmBiT	30µl	W1031
	300µl	W1032
Lumit® Anti-Goat Ab-LgBiT	30µl	W1061
	300µl	W1062
Lumit® Anti-Goat Ab-SmBiT	30µl	W1071
	300µl	W1072
Lumit® Immunoassay Cellular System - Starter Kit	200 assays	W1220
Lumit® Immunoassay Cellular System - Set 1	100 assays	W1201
	1,000 assays	W1202
	10,000 assays	W1203
Lumit® Immunoassay Cellular System - Set 2	100 assays	W1331
	1,000 assays	W1332
	10,000 assays	W1333
Lumit® Immunoassay Lysis and Detection Kit	100 assays	W1231
	1,000 assays	W1232
	10,000 assays	W1233

8. Summary of Changes

The following changes were made to the 2/26 revision of this document:

1. Corrected numbers in Figure 5, Panel A.
2. Corrected measurements in Figure 11, Panel A.
3. Added a patent statement and updated the current patent statement.

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[b]U.S. Pat. Nos. 9,797,889, 9,797,890, 10,107,800 and 11,493,504; European Pat. No. 2970412; Japanese Pat. Nos. 7280842 and 7532562; and other patents and patents pending.

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

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