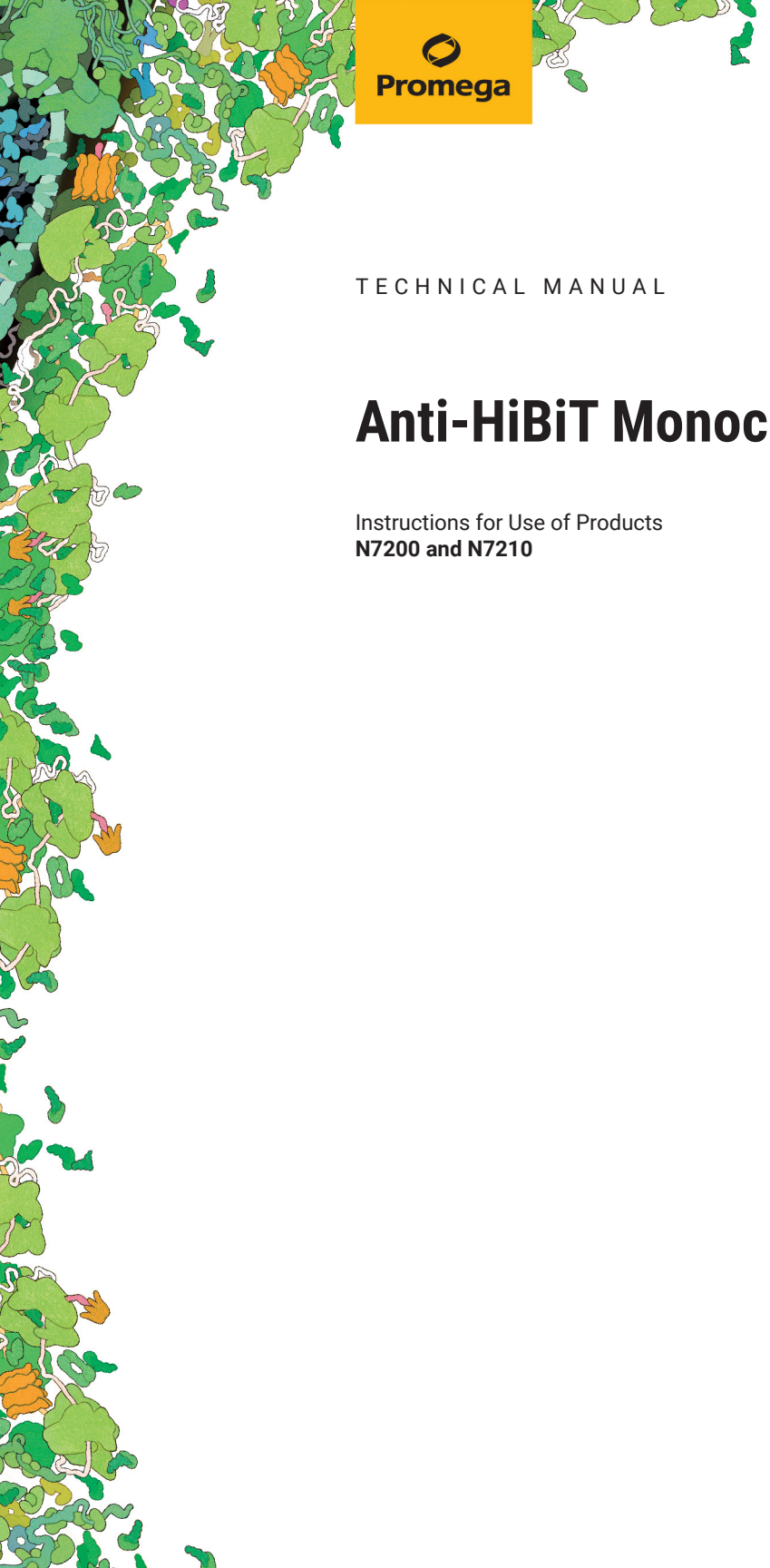

TECHNICAL MANUAL

Anti-HiBiT Monoclonal Antibody

Instructions for Use of Products
N7200 and N7210

4/23
TM719

Anti-HiBiT Monoclonal Antibody

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 High BiT (HiBiT) protein tag is an 11-amino-acid peptide that binds with high affinity to Large BiT (LgBiT) in NanoLuc® Binary Technology (NanoBiT®) to reconstitute NanoBiT® Luciferase, a bright, luminescent enzyme (1,2). Proteins tagged with HiBiT can be easily quantified by homogeneous luminescent assays in multiple formats. Because of its small size and sensitive detection, HiBiT makes an ideal tag for insertion by genome-editing techniques like CRISPR/Cas9, resulting in endogenous expression of HiBiT-tagged proteins (3). The HiBiT tag can also be added to proteins using available HiBiT cloning vectors or directly to existing protein expression constructs by PCR-based or gene synthesis methods. The sequence and rights to synthesize the HiBiT tag can be obtained by reviewing and accepting the Terms and Conditions of Use at: **www.promega.com/HiBiT-Synthesis**

Anti-HiBiT Monoclonal Antibody[a] is a potent and specific mouse monoclonal antibody (mAb) that binds to the HiBiT tag, enabling applications such as immunoblotting, immunofluorescence, immunoprecipitation and fluorescence-activated cell sorting (FACS). The antibody binds to HiBiT with high affinity, with a K_D in solution of approximately 6pM. Because of this high affinity and low background binding, many HiBiT-tagged proteins can be detected at endogenous expression levels.

Anti-HiBiT Monoclonal Antibody can be used in standard protocols for antibody-based applications. This technical manual includes example protocols and representative data for immunoblotting, immunofluorescence, immunoprecipitation and FACS.

Table 1. Anti-HiBiT Monoclonal Antibody Specifications.

Concentration	1.0mg/ml
Storage Buffer	Phosphate-buffered saline (PBS, pH 7.3) with 50% glycerol
Conjugate	Unconjugated
Clone number	30E5
Host Species	Mouse
Antibody Form	Purified immunoglobulin
Isotype	IgG2c with kappa light chain
Purification method	Protein A resin
Immunogen	Synthetic HiBiT peptide

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Anti-HiBiT Monoclonal Antibody	**100µg**	**N7200**
	5 × 100µg	**N7210**

Storage Conditions: Anti-HiBiT Monoclonal Antibody is provided as a sterile-filtered solution in PBS with 50% glycerol without sodium azide. The antibody is shipped frozen on dry ice. Upon receipt, thaw the antibody and centrifuge briefly to collect any solution present on the tube sides or cap. Store the antibody at –30°C to –10°C, where it will remain a liquid.

3. Immunoblotting

3.A. General Considerations

The high affinity and low background binding of the Anti-HiBiT Monoclonal Antibody enable detection of low levels of HiBiT-tagged proteins. Using standard HRP-conjugated secondary antibodies and chemiluminescent substrates, HiBiT-tagged proteins can be detected above background at levels of 1 picogram or less in mammalian cell lysates. Higher-sensitivity chemiluminescent substrates may allow detection of even lower amounts of HiBiT-tagged protein, although background signal from the membrane or cross-reactive bands will also be increased, so optimizing antibody concentrations may be required. Immunoblotting with the Anti-HiBiT Monoclonal Antibody should generally detect most HiBiT-tagged proteins expressed at endogenous levels in CRISPR-derived cell lines.

The Nano-Glo® HiBiT Blotting System (Cat.# N2410) is an alternative antibody-free blotting method for HiBiT-tagged proteins that relies upon complementation with the LgBiT subunit in the reagent to specifically generate the NanoBiT complex on the membrane only at the HiBiT band. Because of the very low bioluminescence background of HiBiT blotting, this system may demonstrate greater sensitivity (higher signal:background ratio) than immunoblotting in some cases, especially if long exposure times are possible to integrate a signal that may be dimmer than chemiluminescent HRP substrates.

Materials to Be Supplied by User

- gel transfer system (e.g., iBlot™ 2 Gel Transfer System, Thermo Fisher Scientific, Cat.# IB21001)
- PVDF or nitrocellulose membrane (e.g., iBlot™ 2 Transfer Stacks, PVDF, Thermo Fisher Scientific Cat.# IB24001 or iBlot™ 2 Transfer Stacks, nitrocellulose, Thermo Fisher Scientific, Cat.# IB23001)
- Tris-buffered saline solution (25mM Tris-HCl, 150mM NaCl, pH 7.5)
- Tween® 20, Molecular Biology Grade (e.g., Cat.# H5152)
- nonfat dry milk (e.g., Research Products International, Cat.# M17200-50)
- HRP-conjugated anti-mouse secondary antibody (Anti-Mouse IgG (H+L), HRP Conjugate, Cat.# W4021)
- ECL Western Blotting Substrate (Cat.# W1001)
- chemiluminescence imaging system

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3.B. Example Protocol

1. After SDS-PAGE, transfer proteins to PVDF or nitrocellulose membrane, for example by using the iBlot™ 2 Gel Transfer System.

   Note: If desired, dry PVDF membranes on filter paper (e.g., overnight). Before proceeding, wet the PVDF membrane for a few seconds in methanol.

2. Rinse the membrane in TBST buffer [Tris-buffered saline + 0.1% (v/v) Tween® 20].

3. Block the membrane in blocking buffer [TBST + 5% (w/v) nonfat dry milk (NFDM)] for 1 hour on a rocking platform at room temperature.

   Note: The protocol has been optimized for blocking with NFDM. Use of other blocking reagents, such as bovine serum albumin or SuperBlock™ Blocking Buffer, may result in higher levels of membrane background or cross-reactive bands.

4. Dilute the Anti-HiBiT Monoclonal Antibody to 1µg/ml in blocking buffer. Replace the blocking buffer on the membrane with this primary antibody solution and incubate with rocking overnight at 4°C.

   Notes:

   a. Lowering the concentration to 0.2µg/ml may decrease the intensity of cross-reactive bands in some conditions. Optimizing antibody concentrations may improve the signal-to-background ratio. When using more sensitive substrates (e.g., SuperSignal™ West Femto, Thermo Fisher Scientific, Cat.# 34095), dilute the antibody further, to 0.04–0.2µg/ml based on the manufacturer's recommendations.

   b. The primary antibody can also be incubated with the membrane for 2 hours at room temperature. Shorter incubation at higher temperature may decrease the intensity of the HiBiT signal compared to overnight incubation at 4°C, but it may also decrease any undesired background signal.

5. Wash 5X with TBST over approximately 15 minutes.

6. Dilute HRP-conjugated anti-mouse secondary antibody (Anti-Mouse IgG (H+L), HRP Conjugate, Cat.# W4021) 2,500- to 5,000-fold into blocking buffer or dilute according to the manufacturer's recommendations. Incubate membrane with rocking at room temperature for 1 hour.

   Note: The most sensitive detection of very small amounts of HiBiT-tagged protein may be limited by the background of the labeled secondary antibody. If possible, dilute the secondary antibody to a concentration that does not produce significant signal in the absence of primary antibody. If using a more sensitive substrate, the secondary antibody should be diluted to a lower concentration according to manufacturer's recommendations (e.g., 0.01µg/ml).

7. Wash 6X with TBST over approximately 30 minutes.

8. Add ECL Western Blotting Substrate to just cover the membrane and incubate for 1 minute.

   Note: For very low levels of protein, a more sensitive substrate can be used with lowered primary and secondary antibody concentrations (e.g., SuperSignal™ West Femto Maximum Sensitivity Substrate, Thermo Fisher Scientific Cat.# 34095).

9. Remove the membrane from the substrate solution, blot the excess liquid on an absorbent towel and place between two clear plastic sheets.

10. Image the membrane with a chemiluminescence imager or with film, using an appropriate exposure time.

3.C. Example Data

Figure 1 demonstrates sensitive detection of low amounts of HiBiT-tagged protein with minimal cross-reactivity from other proteins in a mammalian cell lysate.

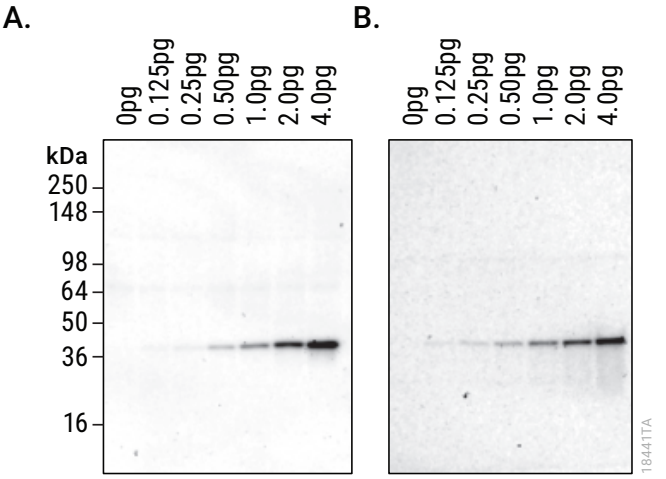

Figure 1. Sensitivity and specificity of immunoblotting with the Anti-HiBiT Monoclonal Antibody compared to the Nano-Glo® HiBiT Blotting System. HaloTag®-HiBiT protein (4; HiBiT Control Protein, Cat.# N3010) was serially diluted into K562 mammalian cell lysate (Mass Spec Compatible Human Protein Extract, Intact; Cat.# V6941) to generate samples of 0.125–4pg of tagged protein diluted into 10µg of lysate protein. After SDS-PAGE, the gel was transferred to a PVDF or nitrocellulose membrane, and immunoblotting was performed using the iBlot™ 2 Gel Transfer Device (Thermo Fisher Scientific, Cat.# IB21001) with 1µg/ml of primary antibody, 0.2µg/ml of HRP-conjugated secondary antibody (Anti-Mouse IgG (H+L), HRP Conjugate, Cat.# W4021) and ECL Western Blotting Substrate (Cat.# W1015). **Panel A.** Anti-HiBiT Monoclonal Antibody immunoblot on PVDF, exposed for 60 minutes and scaled 1,200–15,000. **Panel B.** For comparison, a duplicate gel was transferred to a nitrocellulose membrane to perform the Nano-Glo® HiBiT Blotting Assay (Cat.# N2410), which relies on enzyme complementation to generate a specific bioluminescent signal from HiBiT-tagged proteins on the membrane. The HiBiT blot on nitrocellulose was exposed for 60 minutes and scaled 1,500–5,000. Scaling for both 16-bit images was set from below the membrane background to above the intensity of the 2pg band.

Compared to immunoblotting, Nano-Glo® HiBiT Blotting is often a bit dimmer, but with very few nonspecific bands in mammalian lysates. Immunoblotting with the Anti-HiBiT Monoclonal Antibody allows visualization of subpicogram amounts of HiBiT-tagged proteins in the context of mammalian lysates. Even with a 1-hour exposure, the signal from cross-reactive bands and membrane background remains low, allowing sensitive detection of low amounts of tagged protein.

3.C. Example Data (continued)

Figure 2 shows an immunoblot of lysates from CRISPR-modified cells expressing HiBiT-tagged proteins at endogenous levels.

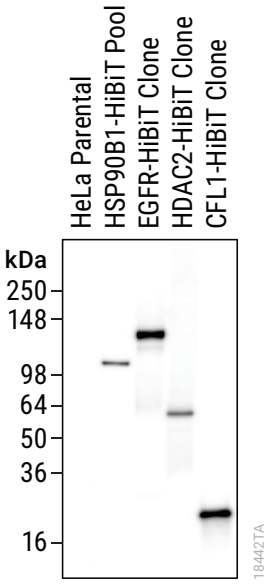

Figure 2. Immunoblotting of lysates from CRISPR-derived cells. Using CRISPR/Cas9 genome-editing technology in HeLa cells, the HiBiT tag was added to the C terminus of HSP90B1, EGFR, HDAC2 and CFL1. The HSP90B1-HiBiT cells represent the CRISPR-modified pool containing predominantly HiBiT-negative cells, while the other three samples represent isolated HiBiT-positive clones. A 4–20% SDS-PAGE gradient gel was run with 10µg of cell lysate, transferred to a PVDF membrane using the iBlot™ 2 Gel Transfer Device (Thermo Fisher Scientific Cat.# IB21001) and immunoblotted as described in Section 3.B with a 5-minute exposure. **Lane 1.** Parental HeLa cells. **Lane 2.** HSP90B1-HiBiT pool (93kDa). **Lane 3.** EGFR-HiBiT clone (135kDa). **Lane 4.** HDAC2-HiBiT clone (56kDa). **Lane 5.** CFL1-HiBiT clone (19kDa).

4. Immunofluorescence

4.A. General Considerations

Anti-HiBiT Monoclonal Antibody can be used to visualize the localization of HiBiT-tagged proteins by immunofluorescence microscopy. While the affinity and specificity of the antibody promote sensitive detection of many endogenously expressed HiBiT-tagged proteins, fluorescence microscopy can be about two orders of magnitude less sensitive than a bioluminescent plate-based assay like the Nano-Glo® HiBiT Lytic Detection System (Cat.# N3030). Therefore, cell lines in which the luminescent signal is less than a few hundred-fold over that of the parental cell line not expressing HiBiT may have fluorescent signals in the microscope that are close to background, especially for proteins with diffuse localization. Cells expressing LgBiT may show greatly decreased staining of HiBiT because of competition between the antibody and LgBiT subunit for binding to HiBiT (see Section 8).

Materials to Be Supplied by User

- imaging plate (e.g., Cellvis, Cat.# P96-1.5H-N)
- paraformaldehyde (e.g., Electron Microscopy Sciences, Cat.# 15710)
- phosphate-buffered saline (e.g., DPBS, GIBCO™ Cat.# 14190)
- Tween® 20, Molecular Biology Grade (e.g., Cat.# H5152)
- Triton® X-100 (e.g., Cat.# H5141)
- normal goat serum (e.g., Abcam, Cat.# ab7481)
- fluorescently-labeled anti-mouse secondary antibody (e.g., Thermo Fisher Scientific Cat.# A-21235)
- fluorescence microscope (e.g., Keyence BZ-X800)
- **optional:** Hoechst stain (e.g., Hoechst 33342 Solution, Thermo Fisher Scientific Cat.# 62249)

4.B. Example Protocol

1. Grow adherent cells in glass-bottom imaging plates.

   Note: One or more of the following controls may be useful for determining the amount of HiBiT-specific signal or optimizing staining conditions:

   - Parental cells lacking HiBiT expression, stained with both primary and secondary antibodies, represent all sources of fluorescence background and are the best comparison for determining how much of the signal in the HiBiT-expressing cell line is specific to the HiBiT-tagged protein.
   - Parental or HiBiT-expressing cells, stained with secondary antibody alone, represent the fluorescent background from the labeled secondary antibody. If this background is similar to the above control, then the Anti-HiBiT Monoclonal Antibody concentrations used are not causing increased background. Lowering the concentration of the secondary antibody may reduce background and enable more sensitive detection.
   - Parental or HiBiT-expressing cells, unstained, represent the autofluorescence background, which may be significant in some channels. Comparison to the control above can determine how much fluorescent background is coming from the labeled secondary antibody.

4.B. Example Protocol (continued)

2. Remove medium, wash once in PBS and fix for 10 minutes at room temperature with 4% paraformaldehyde diluted in PBS.

   Note: Cells fixed with organic solvents like methanol can also be stained with Anti-HiBiT Monoclonal Antibody.

3. Wash 3X with PBS over 5 minutes.
4. Permeabilize cells with PBS + 0.2% (v/v) Triton® X-100 for 10 minutes at room temperature.
5. Wash 3X with PBS over 5 minutes.
6. Block with PBST (PBS + 0.05% Tween® 20) + 5% (v/v) normal goat serum (NGS) for 1 hour at room temperature.

   Note: We recommend blocking with serum from the species in which the secondary antibody was raised.

7. Remove block and add 1µg/ml of Anti-HiBiT Monoclonal Antibody diluted in blocking buffer (PBST + 5% NGS). Incubate for 2 hours at room temperature.

   Note: The antibody can also be incubated overnight at 4°C, but this may increase the amount of nonspecific background.

8. Wash 3X with PBST, 5 minutes per wash.
9. Incubate with a 1:1,000 dilution of anti-mouse Alexa Fluor® 647-conjugated goat secondary antibody (2µg/ml final concentration) or your secondary antibody of choice, diluted in blocking buffer. Incubate for 1 hour at room temperature in the dark.

   Note: When working with cells expressing low levels of HiBiT-tagged proteins, the signal may approach that of the fluorescence background, which consists of both cellular autofluorescence and secondary antibody background binding. Using the far-red channel may significantly decrease the amount of autofluorescence background present.

10. Wash 3X with PBST, 5 minutes each wash, protecting samples from light.
11. For nuclear staining, add Hoechst stain diluted in PBS (e.g., 1–5µg/ml), incubate for 10 minutes, then wash 3X with PBS.
12. Image on a fluorescence microscope with appropriate filters.

4.C. Example Data

Figure 3 shows an example of imaging HiBiT-tagged proteins expressed at endogenous levels in CRISPR-derived HeLa cells.

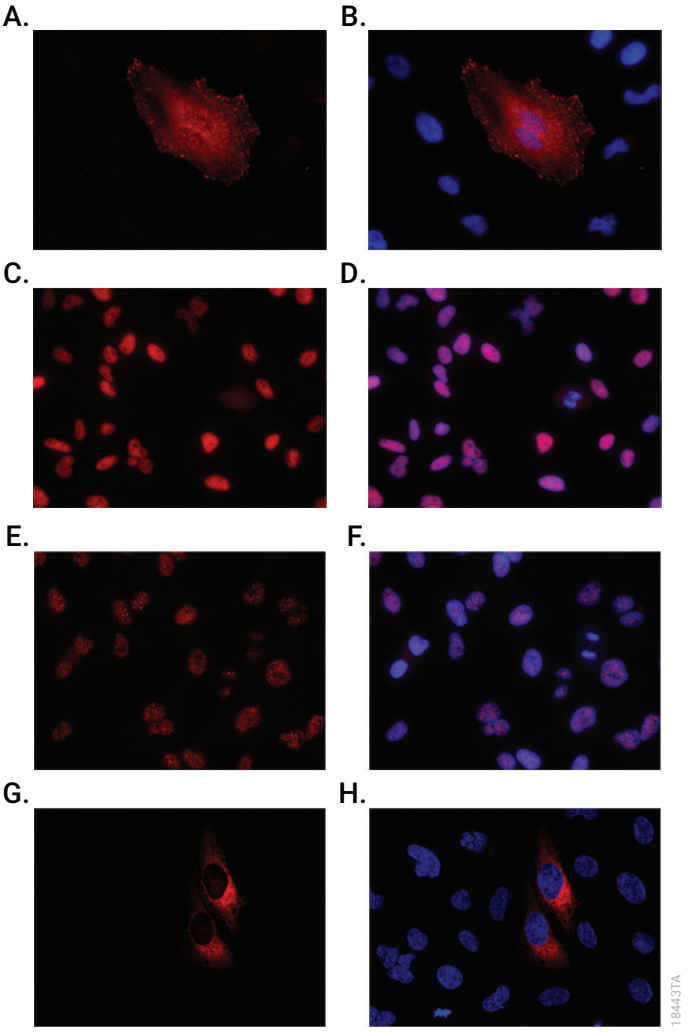

Figure 3. Immunofluorescence imaging of CRISPR-modified HeLa clones or cell pools expressing HiBiT-tagged proteins at endogenous levels. Using CRISPR/Cas9 genome-editing technology in HeLa cells, the HiBiT tag was added to the C terminus of VCL (**Panels A** and **B;** focal adhesions, membrane, cytoskeleton); HDAC2 (**Panels C** and **D;** nucleus); SMARCA4 (**Panels E** and **F;** nucleoli fibrillar centers); and HSP90B1 (**Panels G** and **H;** endoplasmic reticulum, visualized with structured illumination). For HDAC2 and SMARCA4, HiBiT-positive clones were isolated. Fixed cells were immunostained (as described in Section 4.B), with the HiBiT-negative cells in the VCL and HSP90B1 pools demonstrating low background fluorescence. Fixed cells were stained with Anti-HiBiT Monoclonal Antibody and Alexa Fluor® 647-labeled anti-mouse secondary antibody (Thermo Fisher Scientific Cat.# A21235) and imaged on a Keyence BZ-X800 fluorescence microscope. The overlay with Hoechst nuclear stain (blue) is shown in **Panels B, D, F** and **H**.

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5. Immunoprecipitation

5.A. General Considerations

Anti-HiBiT Monoclonal Antibody can be used for immunoprecipitation to capture, concentrate and purify HiBiT-tagged proteins from cell lysates or other samples. Antibody immobilization on protein G-based resins allows easy sample binding, washing and elution. Proteins can be eluted with low pH buffers or by heating with SDS loading buffer, but note that this will cause antibody elution and may denature the HiBiT fusion protein. Eluted antibody can interfere with some downstream applications, such as immunoblotting of samples with anti-mouse secondary antibodies. As an antibody-free method, the Nano-Glo® HiBiT Blotting System (Cat.# N2410) is a good way to visualize HiBiT-tagged proteins in the eluate.

When more gentle elution conditions are required, competition with synthetic HiBiT or DrkBiT Peptide can be used. The DrkBiT Peptide is a variant of HiBiT that binds to the LgBiT subunit to form a nonluminescent complex. Even though DrkBiT has lower affinity for the antibody than does HiBiT Peptide, the concentrations used are well above the K_D so that it can effectively block re-association of HiBiT-tagged proteins that dissociate from the immobilized antibody. Use of either HiBiT or DrkBiT Peptide for elution will interfere with downstream HiBiT luminescent assays, unless the peptides are first separated (e.g., by SDS-PAGE). Due to the slow dissociation rate of HiBiT/mAb complexes (see Section 8), elution with peptide may require relatively long incubation periods (e.g., overnight).

Materials to Be Supplied by User

- magnetic protein G resin (e.g., Cytiva Protein G Mag Sepharose, Cat.# 28944008)
- Tris-buffered saline solution (25mM Tris-HCl, 150mM NaCl, pH 7.5)
- Tween® 20 (Cat.# H5152)
- Mammalian Lysis Buffer (Cat.# G9381)
- RQ1 RNase-Free DNase (Cat.# M6101)
- Protease Inhibitor Cocktail (Cat.# G6521)
- magnetic stand (e.g., MagneSphere® Technology Magnetic Separation Stand, Cat.# Z5342)
- **optional:** SDS loading buffer
- **optional:** low-pH elution buffer (100mM glycine-HCl, pH 2.5)
- **optional:** neutralization buffer (2M Tris-HCl, pH 7.5)
- **optional:** synthetic HiBiT or DrkBiT Peptide (enquire at: **www.promega.com/c/global/forms/contact-tailored-rd-solutions-form/**)

5.B. Example Protocol

1. Wash protein G resin with TBST (Tris-buffered saline plus 0.1% Tween 20) and resuspend to a 20% slurry.
2. Dispense 20µl of 20% resin to each tube.
3. Add 2µg of Anti-HiBiT Monoclonal Antibody to each tube and bind for 10–20 minutes at room temperature. Precipitate beads with magnet and remove supernatant. Remove from magnet and wash beads with 1ml of TBST by gently pipetting. Return tube to magnet and remove wash solution.

   Note: Antibody can be bound to the resin in bulk, followed by dispensing of the slurry into multiple tubes. You can also add the antibody to samples containing the HiBiT-tagged protein, then add the protein G resin.

4. Generate HiBiT-containing samples, e.g., create cell lysates in Mammalian Lysis Buffer supplemented with RQ1 RNase-Free DNase and Protease Inhibitor Cocktail. Clear lysate by centrifuging for 10 minutes at 16,000 × *g* at 4°C and transferring supernatant to a new tube.
5. Add a sample to each tube, e.g., 200µl of cleared cell lysate.
6. Incubate at 4°C overnight, with end-over-end rotation.

   Note: Depending on the application, incubation can also be performed at room temperature for 2 hours or as optimized for your experimental conditions.
7. Precipitate resin using a magnet.
8. Remove supernatant to a new tube for later analysis if desired.
9. Wash 3X with 1ml of TBST.
10. Remove wash buffer and add an elution buffer, such as **one** of the following:
    - Add 50µl of SDS loading buffer and heat at 70°C for 10 minutes.
    - Add 50µl of 100mM glycine-HCl (pH 2.5) and incubate at room temperature for 5–10 minutes with agitation or end-over-end rotation. Neutralize the eluate with a 20% volume of 2M Tris-HCl (pH 7.5).
    - Add 50µl of 10µM HiBiT or DrkBiT Synthetic Peptide in TBST and incubate overnight at 4°C with end-over-end rotation.

    Note: If using HiBiT or DrkBiT, incubation at room temperature and/or for shorter amounts of time is also possible. The extent of elution will depend on the dissociation rate of HiBiT from the immobilized antibody, which may differ for different fusion proteins.
11. Load samples onto the gel and analyze by SDS-PAGE.

5.C. Example Data

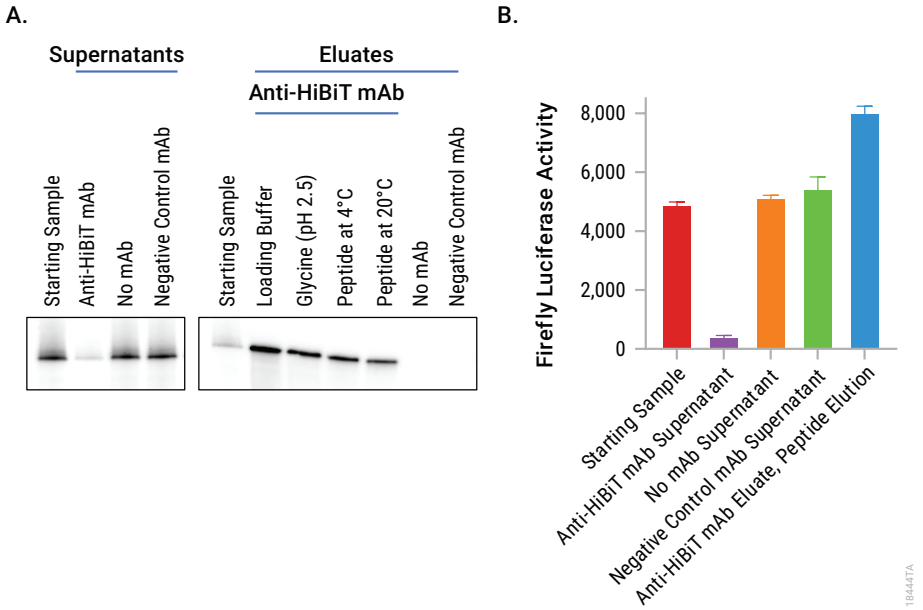

Figure 4. Immunoprecipitation of HiBiT-tagged firefly luciferase (Fluc-HiBiT) from transiently transfected HEK293 cells. Starting samples consisted of 1ml of lysate containing 2 × 10^6 cells/ml in Mammalian Lysis Buffer (Cat.# G9381) supplemented with Protease Inhibitor Cocktail (Cat.# G6521) and RQ1 RNase-Free DNase (Cat.# M6101). Protein G Mag Sepharose (Cytivia Cat.# 28944008) was incubated with Anti-HiBiT Monoclonal Antibody, no antibody or a negative control antibody (anti-Myc mAb, clone 9E10). Cleared lysate samples were incubated with the sepharose overnight at 4°C. Samples were eluted by heating with SDS loading buffer, but replicate tubes containing the Anti-HiBiT Monoclonal Antibody were also eluted with glycine (pH 2.5), overnight DrkBiT Peptide incubation at 4°C or overnight DrkBiT Peptide incubation at room temperature. **Panel A** shows analysis of samples using the Nano-Glo® HiBiT Blotting System (Cat.# N2410) to avoid interference by eluted antibody. The left panel is scaled to lower intensities to illustrate that Fluc-HiBiT is cleared effectively by Anti-HiBiT Monoclonal Antibody, but not the negative controls. The right panel is scaled to higher intensities to demonstrate increased concentrations of Fluc-HiBiT in the eluates with Anti-HiBiT Monoclonal Antibody, but not the negative controls. In **Panel B** firefly luciferase activity of Fluc-HiBiT was measured using the ONE-Glo™ Assay (Cat.# E6110), highlighting the >90% clearance of Fluc-HiBiT from the supernatant and the ability to maintain the enzyme activity of the fusion partner by eluting with competing peptide.

Figure 5 shows immunoprecipitation of HiBiT-tagged proteins expressed at endogenous levels in CRISPR-modified cells.

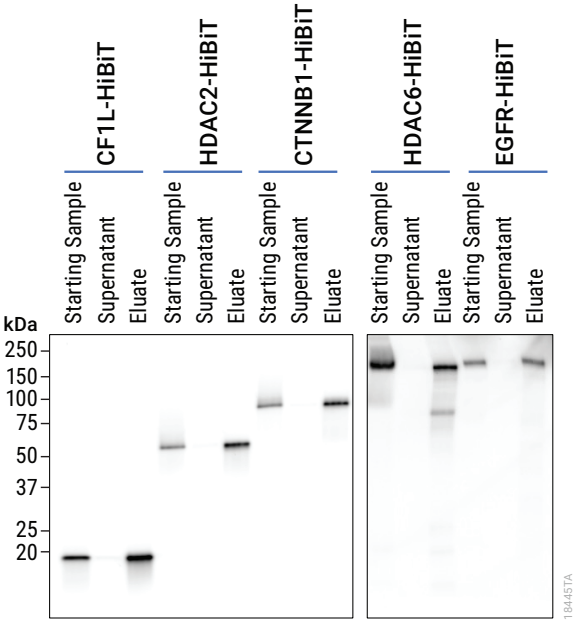

Figure 5. Immunoprecipitation of HiBiT-tagged proteins from CRISPR-modified HeLa cells. Using CRISPR/Cas9 genome-editing technology in HeLa cells, the HiBiT tag was added to the C terminus of CFL1, HDAC2, CTNNB1, HDAC6 and EGFR. The standard protocol in Section 5.B was used to immunoprecipitate the proteins, and samples were analyzed to monitor protein clearance in the supernatant and protein recovery in the eluate. Starting samples of cleared cell lysate (0.15ml; 5 × 10^6 cells/ml) were immunoprecipitated by binding overnight at 4°C and eluting in 50µl of SDS loading buffer. Equivalent volumes of each original lysate sample, supernatant and eluate were analyzed with the Nano-Glo® HiBiT Blotting System (Cat.# N2410). Before gel loading, the three samples from each cell line were further diluted into SDS loading buffer to make the HiBiT levels more similar across the membrane: CFL1 (4X dilution), HDAC2 (4X dilution), CTNNB1 (4X dilution), HDAC6 (no dilution), EGFR (20X dilution). The images on the left and right represent 4- and 20-minute exposures, respectively.

5.C. Example Data (continued)

Figure 6 demonstrates the results of purification of HiBiT-tagged maltose-binding protein (MBP-HiBiT-His6) from *E. coli* lysates.

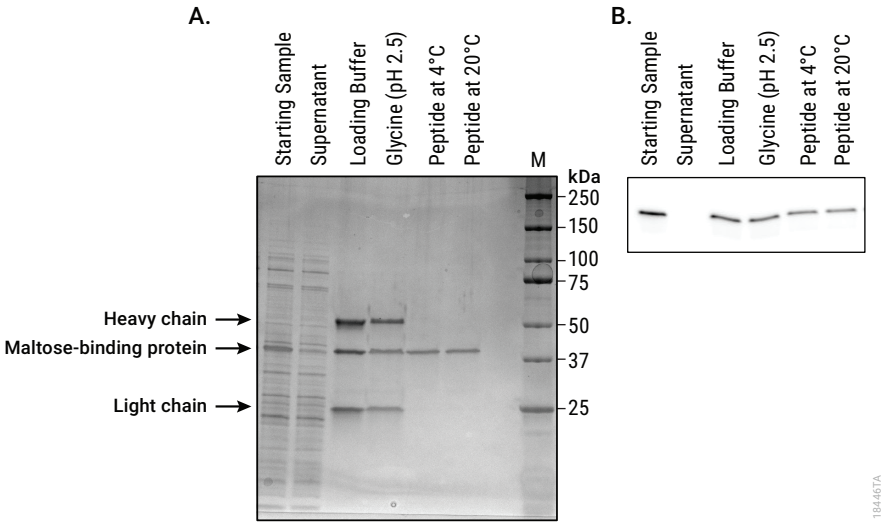

Figure 6. Purification of HiBiT-tagged maltose-binding protein (MBP-HiBiT-His6) from *E. coli* lysate. Lysate from *E. coli* transformed with an expression construct for MBP-HiBiT-His6 was diluted 50-fold into lysis buffer to generate the starting sample. After immunoprecipitation with Anti-HiBiT Monoclonal Antibody overnight at 4°C, the washed resin was divided into separate tubes and eluted with either SDS loading buffer, glycine at pH 2.5, overnight DrkBiT Peptide incubation at 4°C or overnight DrkBiT Peptide elution at room temperature. **Panel A.** Silver staining demonstrates clearance of MBP-HiBiT-His6 from the sample and purification from lysate proteins in the eluate. Elution with SDS loading buffer or at low pH caused co-elution of the Anti-HiBiT Monoclonal Antibody as seen by the presence of heavy and light chains on the gel. **Panel B.** A separate gel was analyzed with the Nano-Glo® HiBiT Blotting System (Cat.# N2410) to show effective clearance of MBP-HiBiT-His6 from the sample and recovery of the protein in the four eluates.

6. Fluorescence-Activated Cell Sorting (FACS) with Live Cells

6.A. General Considerations

The HiBiT peptide tag can be fused to plasma membrane proteins via an extracellular terminus or by insertion into a surface loop. In these cases, antibody staining of living cells can be used to quantify cell-surface expression or to isolate clonal cell lines expressing the HiBiT-tagged protein. FACS analysis of cells expressing intracellular HiBiT will require fixation, as discussed in Section 7.

Materials to Be Supplied by User

- phosphate-buffered saline (e.g., DPBS, GIBCO™, Cat.# 14190)
- fetal bovine serum (FBS)
- bovine serum albumin (e.g., Sigma, Cat.# A7979)
- Versene (Thermo Fisher Scientific Cat.# 15040066)
- fluorescently labeled secondary antibody (e.g., FITC anti-mouse IgG Antibody, BioLegend Cat.# 406001)
- flow cytometer (e.g., BD LSRFortessa™ X-20 Cell Analyzer)
- polystyrene centrifuge tube with filter cap (e.g., Corning® Falcon® Cat.# 352235)

6.B Example Protocol

Include an isotype control antibody (IgG2c, kappa) and/or parental cells lacking expression of a HiBiT fusion protein as negative controls.

1. Prepare FACS buffer by combining DPBS with 1% (v/v) FBS. Alternatively, 1% (w/v) BSA can be used instead of FBS if serum-mediated receptor internalization is a concern.

Harvest and Count Cells

2. Dislodge adherent cells with Versene or similar mild dissociation solution. Do not use trypsin to dislodge cells due to proteolytic cleavage of the HiBiT sequence. Gently scrape adherent cells from the surface of the flask, if needed.
3. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.
4. Gently resuspend cell pellet in FACS buffer to 10^6 cells/ml.
5. Add 1ml of cell suspension per tube.
6. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.
7. Gently resuspend cell pellets using 1ml of FACS buffer per tube.
8. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.

Add Primary Antibody

9. Dilute the Anti-HiBiT Monoclonal Antibody and the isotype control to 1µg/ml in FACS buffer.
10. Gently resuspend pellets by adding 50µl of primary antibody solution per tube.
11. Incubate tubes on ice for 30 minutes with periodic mixing.

6.B Example Protocol (continued)

Wash Cells

12. Add 1ml of FACS buffer per tube.
13. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.
14. Gently resuspend pellets using 1ml of FACS buffer per tube.
15. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.

Add Secondary Antibody

16. Dilute secondary antibody to recommended concentration using FACS buffer.
17. Gently resuspend pellets using 50µl of secondary antibody solution per tube.
18. Incubate on ice for 30 minutes in the dark with periodic mixing.

Wash Cells

19. Add 1ml of FACS buffer per tube.
20. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.
21. Gently resuspend pellets using 1ml of FACS buffer per tube.
22. Centrifuge at 300 × *g* for 5 minutes and aspirate supernatant.
23. Gently resuspend pellets using 500µl of FACS buffer per tube.
24. Filter cells into a 5ml polystyrene tube with filter cap.
25. Analyze by flow cytometry.

6.C. Example Data

Figure 7 demonstrates live-cell FACS of HeLa and HeLa/HiBiT-EGFR cells.

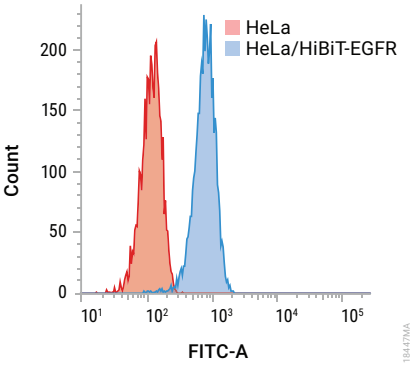

Figure 7. Live-cell FACS with HeLa and HeLa/HiBiT-EGFR cells. CRISPR/Cas9 was used to create a heterozygous knock-in of HiBiT at the extracellular N terminus of EGFR in HeLa cells. A clonal HiBiT-EGFR cell line and the parental HeLa cells were stained using the protocol in Section 6.B, with BSA replacing FBS in the FACS buffer to prevent receptor internalization. HeLa parental cells and HeLa/HiBiT-EGFR cells were stained with 1µg/ml of Anti-HiBiT Monoclonal Antibody, followed by a 1:100 dilution of FITC anti-mouse IgG Antibody (BioLegend Cat.# 406001) as the secondary antibody. Flow cytometry was performed on a BD LSRFortessa™ X-20 Flow Cytometer using the FITC-A filter setting.

7. Fluorescence-Activated Cell Sorting with Fixed Cells

7.A. General Considerations

To quantify expression of intracellular HiBiT-tagged proteins, HiBiT assays like the Nano-Glo® HiBiT Lytic Detection System (Cat.# N3030) or live-cell assays involving expression of LgBiT inside the cell are generally preferred. However, FACS analysis of fixed, permeabilized cells that have been stained with Anti-HiBiT Monoclonal Antibody can be used to measure the protein expression distribution in a cell population or to correlate those expression levels to another protein.

Materials to Be Supplied by User

- Cyto-Fast™ Fix/Perm Buffer Set (BioLegend Cat.# 426803)
- phosphate-buffered saline (DPBS, GIBCO™ Cat.# 14190)
- FACS buffer (DPBS + 2% FBS)
- fetal bovine serum (FBS)
- fluorescently-labeled secondary antibody (e.g., FITC anti-mouse IgG Antibody, BioLegend Cat.# 406001)
- flow cytometer (e.g., BD LSRFortessa™ X-20 Cell Analyzer)
- polystyrene centrifuge tube with filter cap (e.g., Corning® Falcon® Cat.# 352235)

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7.B. Example Protocol

The following example uses the Cyto-Fast™ Fix/Perm Buffer Set. Alternative reagents or protocols for fixation or permeabilization are also expected to work. Include an isotype control antibody (IgG2c, kappa) and/or parental cells lacking expression of a HiBiT fusion protein in your experiment as negative controls.

Note: FACS buffer used in Section 7.B differs from the FACS buffer used in Section 6.B.

1. Trypsinize adherent cells and resuspend in six volumes of growth medium. If cells are expressing an extracellular HiBiT tag, do not use trypsin. Instead use a gentler cell dissociation reagent like Versene.
2. Pellet 10^6 cells and wash 1X in 1ml of DPBS.
3. Resuspend the cell pellet in 100µl DPBS.
4. Dilute the Cyto-FAST™ Perm Wash solution (a component of Cyto-Fast™ Fix/Perm Buffer Set) 10X using deionized water.
5. Add 100µl of cell suspension per 15ml conical tube.
6. Add 150µl of Cyto-Fast™ Fix/Perm Buffer per tube and mix.
7. Incubate for 20 minutes at room temperature.
8. Add 1ml of 1X Cyto-Fast™ Perm Wash solution per tube.
9. Centrifuge at 350 × *g* for 5 minutes and aspirate supernatant.
10. Repeat Steps 8 and 9.
11. Dilute Anti-HiBiT Monoclonal Antibody and isotype control to 1µg/ml using 1X Cyto-Fast™ Perm Wash Solution. Resuspend cell pellets by adding 100µl of the respective primary antibody solution per tube.
12. Incubate for 20 minutes at room temperature.
13. Add 1ml of Cyto-Fast™ Perm Wash solution per tube and mix.
14. Centrifuge at 350 × *g* for 5 minutes and aspirate supernatant.
15. Resuspend cell pellets by adding 1ml of FACS buffer (DPBS + 2% FBS) per tube and mixing.
16. Centrifuge at 350 × *g* for 5 minutes and aspirate supernatant.
17. Dilute secondary antibody to recommended concentration using FACS buffer. Resuspend cell pellets by adding 100µl of secondary antibody solution per tube.
18. Incubate on ice for 30 minutes in the dark.
19. Add 1ml of FACS buffer per tube and mix.
20. Centrifuge at 350 × *g* for 5 minutes and aspirate supernatants.
21. Resuspend cell pellets by adding 1ml of FACS buffer per tube and mixing.
22. Centrifuge at 350 × *g* for 5 minutes and aspirate supernatants.
23. Resuspend cell pellets by adding 500µl of FACS buffer per tube and mixing.

24. Filter cells into a 5ml polystyrene tube with filter cap (Corning® Falcon® Cat.# 352235).
25. Analyze by flow cytometry.

7.C. Example Data

Figure 8 shows the results of FACS analysis with CRISPR-modified HeLa Cells. CRISPR/Cas9 was used to insert HiBiT at the C terminus of the endogenous locus of CFL1, EGFR, HDAC2, HDAC6 and CASP3 in HeLa cells.

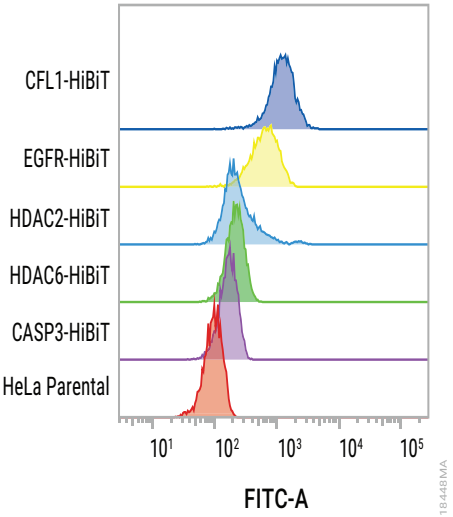

Figure 8. FACS with fixed CRISPR-modified HeLa cells. HeLa parental cells and CRISPR-modified HeLa clones expressing HiBiT-tagged CFL1, EGFR, HDAC2, HDAC6 or CASP3 at endogenous levels were fixed and permeabilized using the Cyto-Fast™ Fix/Perm Buffer Set (BioLegend Cat.# 426803). Cells were stained using the protocol in Section 7.B with 1µg/ml of Anti-HiBiT Monoclonal Antibody, followed by a 1:100 dilution of FITC anti-mouse IgG antibody (BioLegend Cat.# 406001) as the secondary antibody. Flow cytometry was performed on a BD LSRFortessa™ X-20 Flow Cytometer using the FITC-A filter setting.

8. Antibody Binding Affinity and Competition with LgBiT

Anti-HiBiT Monoclonal Antibody binds with high affinity to form a stable complex with HiBiT peptide or HiBiT-tagged proteins. Antibody binding largely blocks productive interaction of HiBiT with the LgBiT subunit, although it is possible that lower-affinity, lower-activity ternary complexes may form. Because binding is mutually exclusive, the antibody affinity can be measured by pre-equilibrating HiBiT/antibody complexes at low concentrations in solution and measuring inhibition of NanoBiT® Luciferase signal upon addition of saturating LgBiT. Extended equilibration of a titration of Anti-HiBiT Monoclonal Antibody with HiBiT peptide suggests a K_D of about 6pM, or about 0.44ng/ml (Figure 9). The HaloTag®-HiBiT fusion protein (HiBiT Control Protein, Cat.# N3010) gave a higher K_D of 22pM or 1.6ng/ml.

8. Antibody Binding Affinity and Competition with LgBiT (continued)

By diluting preformed HiBiT/Anti-HiBiT Monoclonal Antibody complexes into saturating LgBiT subunit, the dissociation rate can be measured from the increasing NanoBiT activity over time. These results suggest a dissociation rate constant (k_d) of around 1.1×10^{-4} second^{-1} for HiBiT (1.7 hour half-life) and 8.2×10^{-5} second^{-1} for HaloTag®-HiBiT protein (2.3 hour half-life). Observed second-order association rate constants are largely consistent with values predicted from the K_D and k_d (4×10^6–2×10^7 M^{-1} second^{-1}). Immobilization of the antibody or HiBiT-tagged protein may change the affinity or kinetics compared to solution.

LgBiT expression may impact Anti-HiBiT Monoclonal Antibody performance because it competes for binding to HiBiT. However, the antibody binds at least two orders of magnitude more tightly to HiBiT than does LgBiT. For immunoprecipitation of HiBiT-tagged proteins from a cell lysate, LgBiT expression in the cell is unlikely to have an effect because of the LgBiT dilution upon lysis, the high concentration of antibody used and the high affinity of the antibody interaction. With immunofluorescence, however, LgBiT expression may have a greater effect because of fixation of preformed HiBiT/LgBiT complexes. HiBiT-tagged proteins may still generate specific signal upon immunostaining, but it will likely be reduced, depending on the level of LgBiT expression. Additionally, the presence of Anti-HiBiT Monoclonal Antibody in a sample can affect quantitation of HiBiT in bioluminescent assays in which LgBiT is a component of the reagent (e.g., the Nano-Glo® HiBiT Lytic Detection System).

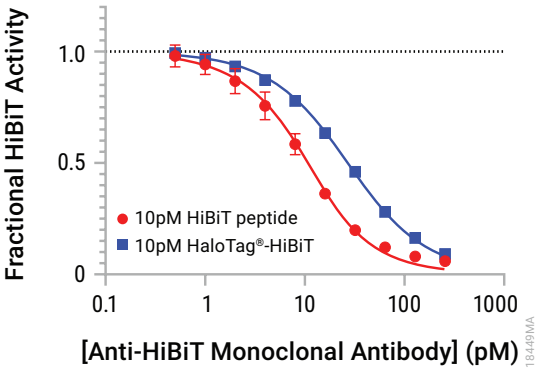

Figure 9. Measuring the affinity of the Anti-HiBiT Monoclonal Antibody by inhibition of NanoBiT® activity. A titration of antibody concentrations was incubated for 40 hours at room temperature with 10pM of either HiBiT peptide or HaloTag®-HiBiT protein to reach equilibrium. The fraction of unbound HiBiT was measured by adding a HiBiT detection reagent containing saturating LgBiT plus furimazine substrate, and measuring the HiBiT luminescence from the NanoBiT® complex. Since the K_D is comparable to the HiBiT concentration used, the data was fit to the equation for tight-binding inhibitors, yielding a value of 6pM for HiBiT peptide and 22pM for HaloTag®-HiBiT protein.

9. Troubleshooting

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

9.A. Immunoblotting

Symptoms	Causes and Comments
High intensity of cross-reactive bands	The intensity of cross-reactive bands in 10µg of mammalian lysate should generally be less than that of 1pg of HaloTag®-HiBiT protein. Nonspecific binding of the Anti-HiBiT Monoclonal Antibody to additional bands might be decreased with one or more of the following: Block with 5% (w/v) nonfat dry milk and use this for both primary and secondary antibody solutions. In some cases, switching to 5% Fetal Bovine Serum as a block has reduced background bands without affecting the HiBiT signal.
	Reduce the concentration of Anti-HiBiT Monoclonal Antibody to 0.2µg/ml or less.
	Perform the primary antibody incubation for a shorter amount of time at a higher temperature (e.g., two hours at room temperature).
HiBiT bands have low signal	Using concentrations of Anti-HiBiT Monoclonal Antibody higher than 1µg/ml will generally not improve the signal:background ratio. Consider the following to increase signal: Use a sensitive chemiluminescent HRP substrate.
	Increase the concentration of labeled secondary antibody.
	Expose the image for longer (e.g,. 30–60 minutes).
	Load more HiBiT-tagged protein (e.g., concentrate the protein by first immunoprecipitating with the Anti-HiBiT Monoclonal Antibody.)
	Incubate the primary antibody overnight at 4°C.
The membrane background signal is high	High background on the membrane itself, as opposed to cross-reactive bands, is likely caused by the secondary antibody. Consider one or more of the following: Lower the concentration of secondary antibody used.
	Try a different batch of secondary antibody.
	Do more extensive washing after secondary antibody incubation (e.g., 6 or more washes over 30 minutes).
	Use a less sensitive substrate or shorten the exposure time.
	Try changing the type of membrane (PVDF vs. nitrocellulose).

Symptoms	Causes and Comments
There are dark blotches on the membrane	This is often caused by dry patches or insufficient washing of a section of the membrane, or the presence of a foreign substance on the membrane. Make sure to only handle membrane with clean forceps from the edges and avoid touching the membrane surface.
	Remove any bits of gel sticking to the membrane.
	Make sure that the membrane is never allowed to dry, use plenty of buffer to cover the membrane and use a platform shaker to provide even washing of the entire surface.
	Centrifuge the secondary antibody stock tube to spin down any aggregates before use.
	Ensure blocking reagent is completely dissolved before use.

9.B. Immunofluorescence

Symptoms	Causes and Comments
Fluorescence background is high	Fluorescence background comes from cellular autofluorescence, nonspecific primary antibody binding and nonspecific secondary antibody binding. Decrease fluorescence background for more sensitive detection of HiBiT as follows: Autofluorescence is generally lower in further-red channels. Consider using a secondary antibody labeled with a far-red dye like Alexa Fluor® 647.
	Fixation with cross-linkers can generate autofluorescence, so use paraformaldehyde instead of glutaraldehyde and keep the fixation time to a minimum.
	Lower the concentration of secondary antibody to the point where it generates negligible signal over autofluorescence background when added to cells in the absence of primary antibody.
	If the secondary antibody is generating minimal background, but there is significant background when both the primary and secondary antibodies are used with parental cells not expressing HiBiT, then nonspecific primary antibody binding is causing background. Lower the concentration of Anti-HiBiT Monoclonal Antibody used in staining of both the parental cell line and the HiBiT-expressing cell line to determine the optimal dilution to reduce background while maintaining signal.
	Increase the number or duration of washes of the primary and secondary antibodies.

Symptoms	Causes and Comments
Fluorescence background is high (continued)	Use 5% normal serum from the species the secondary antibody was raised in for blocking and antibody dilution. Blocking in normal goat serum and then diluting the primary antibody in a BSA solution can generate high background.
The HiBiT signal is low	Fluorescence imaging is intrinsically a less sensitive detection method than HiBiT bioluminescent assays, so if the luminescent signal in the Nano-Glo® HiBiT Lytic Assay is less than 300-fold over that of the parental cells lacking HiBiT, it may be difficult to image the HiBiT-tagged protein unless it has a very specific subcellular localization. Potential ways to increase the signal include: Raising the concentration of the primary and/or secondary antibody. Compare the HiBiT-expressing cell to the parental non-HiBiT cell to determine the optimal concentrations to maximize the signal-to-background ratio. In general, increasing the Anti-HiBiT Monoclonal Antibody concentration above 1µg/ml will not increase the S/B ratio.
	Consider using a secondary antibody with a brighter fluorophore.

9.C. Immunoprecipitation

Symptoms	Causes and Comments
There is low clearance of HiBiT-tagged protein from the supernatant	If a relatively low percentage of HiBiT-tagged protein appears to be immunoprecipitated onto immobilized antibody, consider the following possibilities: The amount of HiBiT-tagged protein may exceed the binding capacity of the immobilized antibody. Increase the amount of antibody used.
	The amount of antibody may exceed the binding capacity of the resin, potentially leading to free antibody in solution. Increase the amount of resin used and wash away unbound antibody before adding the HiBiT-containing sample.
	The cell lysis or buffer conditions may not be optimal. Ensure that cell lysis and protein solubilization is efficient; the high affinity of the HiBiT/mAb interaction helps maintain the interaction even in relatively harsh detergent conditions, like RIPA buffer. If the lysate is too viscous, treat with DNase, but make sure there is no EDTA present during treatment.
	The binding time may not be sufficient. Increase the binding time (e.g., overnight at 4°C).

9.C. Immunoprecipitation (continued)

Symptoms	Causes and Comments
There is low clearance of HiBiT-tagged protein from the supernatant (continued)	The HiBiT tag on the target protein may not be fully accessible (e.g., because it is buried in a protein complex). A lower-than-expected HiBiT bioluminescent signal upon addition of LgBiT may help confirm this. Increasing the stringency of the binding buffer could help make the tag more accessible. Consider increasing the linker length between the target protein and the HiBiT tag or append it to the other protein terminus.
Co-elution of antibody complicates downstream analysis	Elution of immunoprecipitated proteins from Protein A or G resins with low pH buffers or by heating in SDS loading buffer will also cause elution of the antibody. Incubation with competing HiBiT or DrkBiT Peptide will retain the antibody on the resin and elute only HiBiT-tagged proteins.
	Alternatively, the antibody can be covalently immobilized (e.g., by cross-linking or using NHS-activated resin) to prevent elution from the resin.
Low amount of HiBiT-tagged protein in eluate	If the HiBiT-tagged protein was effectively cleared from the supernatant but the amount eluted is low compared to the starting amount, there could be several possibilities: The HiBiT fusion protein dissociated during the wash steps. Because of the stable complex between HiBiT and the mAb, this is unlikely unless a particularly harsh wash buffer was used.
	The protein may have degraded. Add Protease Inhibitor Cocktail (Cat.# G6521) to the lysis buffer and initial sample buffer and, if necessary, to wash or elution buffers.
	Perform all IP steps at 4°C.
	The protein may be unstable or precipitating irreversibly onto the resin. Optimize buffer conditions to maintain protein solubility.
	Perform a second elution step heating with SDS loading buffer to see what protein may still be stuck to the resin.
An expected coprecipitating protein is not observed in the eluate	If a significant amount of HiBiT tagged protein is immunoprecipitated, but an expected binding partner is not observed in the eluate, the protein complex may not be stable and dissociation may be occurring during the binding or washing steps. Use a shorter incubation time to try to preserve the complex.

Symptoms	Causes and Comments
An expected coprecipitating protein is not observed in the eluate (continued)	Optimize buffer conditions to maintain the protein-protein interaction of interest (e.g., adding glycerol to stabilize the complex).
	Perform all IP steps at 4°C.
	The stoichiometry of the protein complex in cells may mean that only a small percentage of the HiBiT-tagged protein is bound to the second protein at the point of lysis. Consider overexpressing the coprecipitating protein.
The eluate appears to contain nonspecifically bound proteins	Increase wash stringency by raising the salt concentration, adding detergents to the wash buffer or increasing the wash volume.
	Elute more specifically using HiBiT or DrkBiT Peptide, rather than SDS Loading buffer or low pH buffers.

9.D. Fluorescence-Activated Cell Sorting

Symptoms	Causes and Comments
The fluorescence signal from the HiBiT-expressing cells is weak	The expression level of the HiBiT-tagged protein may be too low to give a fluorescence signal significantly over background, even if the HiBiT luminescent signal is well over background.
	Optimize the concentrations of primary and secondary antibodies, testing higher concentrations.
	Make sure to use an anti-mouse IgG secondary antibody.
	Make sure the flow cytometer settings (laser, alignment, PMT) are correct for the fluorophore being used.
	Trypsinization of adherent cells could destroy surface antigens. When HiBiT is expressed on the surface, use a gentle cell dissociation method like Versene® solution.
	For fixed cells, ensure that the permeabilization conditions are appropriate for the subcellular localization of the protein.
	Cell-surface target proteins may internalize during staining. Keep samples at 4°C and use ice-cold reagents to reduce internalization.
The fluorescence signal or background is too high	Optimize the concentrations of primary and secondary antibodies, testing lower concentrations.
	Cell debris can cause high background. Gate to exclude low-scatter debris from your analysis.
	Adjust the gain and offset values on the flow cytometer.
	Perform additional washes to remove unbound antibody.

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10. References

1. Dixon, A.S. *et al.* (2016) NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* **11**, 400–8.
2. Hall, M.P. *et al.* (2012) Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS Chem. Biol.* **7**, 1848–57.
3. Schwinn, M.K. *et al.* (2018). CRISPR-mediated tagging of endogenous proteins with a luminescent peptide. *ACS Chem. Biol.* **13**, 467–74.
4. Los, G.V. *et al.* (2008) HaloTag: a novel protein labeling technology for cell imaging and protein analysis. *ACS Chem. Biol.* **3**, 373–82.

11. Related Products

Product	Size	Cat. #
Nano-Glo® HiBiT Blotting System	100ml	N2410
Nano-Glo® HiBiT Lytic Detection System*	10ml	N3030
Nano-Glo® HiBiT Extracellular Detection System*	10ml	N2420
HiBiT Control Protein	100µl	N3010
LgBiT Stable Cell Line	1 each	N2672
LgBiT Expression Vector	20µg	N2681
FuGENE® 4K Transfection Reagent*	1ml	E5911
ViaFect™ Transfection Reagent*	0.75ml	E4981
Mammalian Lysis Buffer	40ml	G9381
Protease Inhibitor Cocktail	1ml	G6521
RQ1 RNase-Free DNase	1,000u	M6101
Anti-Mouse IgG (H+L), HRP Conjugate	300µl	W4021
ECL Western Blotting Substrate*	250ml	W1001
TMB Stabilized Substrate for HRP*	500ml	W1015
Anti-Mouse IgG (H +L), AP Conjugate	100µl	S3721
Western Blue® Stabilized Substrate for Alkaline Phosphatase	100ml	S3841

*Additional sizes are available.

HiBiT Cloning Vectors

Vector Name	Cloning Format	Tag Orientation	Cat.#
pBiT3.1-N [CMV/HiBiT/Blast]	MCS	HiBiT-POI	N2361
pBiT3.1-C [CMV/HiBiT/Blast]	MCS	POI-HiBiT	N2371
pBiT3.1-secN [CMV/HiBiT/Blast]	MCS	IL6-HiBiT-POI	N2381
pFN36K HiBiT CMV-neo Flexi® Vector	Flexi	HiBiT-POI	N2401
pFC37K HiBiT CMV-neo Flexi® Vector	Flexi	POI-HiBiT	N2391
pFN39K secHiBiT CMV-neo Flexi® Vector	Flexi	IL6-HiBiT-POI	N2411