A Peptide Tag for the Simple and Sensitive Bioluminescent Quantification of Proteins

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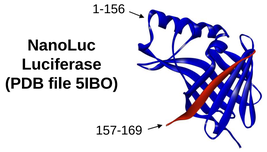
1. Introduction

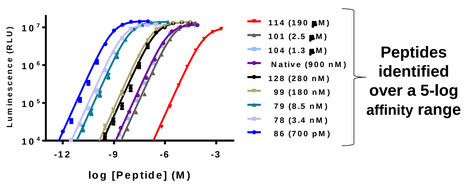
We have developed a multifunctional protein tag utilizing NanoLuc Binary Technology (NanoBiT), a binary complementation system based on NanoLuc luciferase. The tag, High BiT (HiBiT), is only 11 a.a. in length, which minimizes any potential impact on fusion partner function. Expression levels of HiBiT-tagged proteins in mammalian cells are quantified using a lytic detection reagent containing Large BiT (LgBiT), which binds tightly to HiBiT (K_D~1 nM) to generate a bright, luminescent enzyme. The assay provides over seven logs of linear dynamic range with a limit of detection of less than 0.1 attomoles (3 fg of 30 kDa protein), allowing proteins to be quantified at endogenous levels of expression. The assay is compatible with high-throughput screening, using a simple add-mix-read protocol with luminescence measured after 10 minutes. To determine size, HiBiT-tagged proteins can be resolved via SDS-PAGE and quantified on blots at sub-picogram levels by adding a detection reagent containing LgBiT. In contrast to immunodetection, which requires multiple hours owing to blocking, binding and washing steps, HiBiT blotting takes only minutes to perform because signal is generated only where HiBiT binds to LgBiT. In addition, the internalization, secretion, or cell surface expression of HiBiT-tagged proteins can be quantified in less than 5 minutes using a non-lytic detection reagent that contains cell-impermeable LgBiT and furimazine.

2. NanoBiT Technology Overview

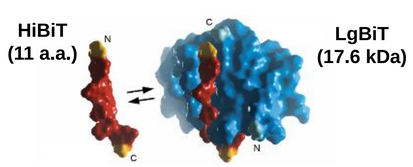


- Numerous split sites were identified with a low affinity for self-association
- Site between 156/157 selected for development
- 1-156 fragment evolved for enhanced structural stability to give Large BiT (LgBiT)

- Numerous peptides screened with LgBiT
- Highest affinity peptide, PEP86, was selected as HiBiT
- Lowest affinity peptide, PEP114, selected for protein:protein interaction assays

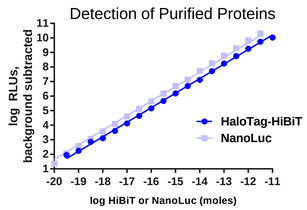


3. HiBiT Technology Overview



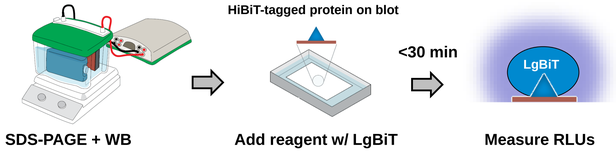
- High-affinity interaction between HiBiT and LgBiT (K_D = 700 pM) drives rapid binding of purified LgBiT in the reagent to HiBiT in the sample
- Interaction generates a bioluminescent enzyme that gives a bright, extended glow-type signal in the presence of furimazine substrate (>10-fold as bright as firefly luciferase)
- HiBiT can be fused to the N- or C-terminus of proteins, or even placed in accessible internal locations
- Multifunctional peptide tag (lytic detection, blotting, or live cell, non-lytic assays)

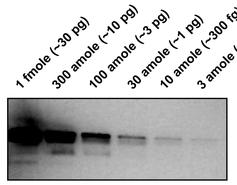
4. Quantification of HiBiT-Tagged Proteins in Cell Lysates



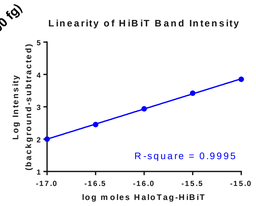
- LgBiT-containing detection reagent that lyses cells
- Homogeneous assay format with measurement 10 minutes after reagent addition and a signal stable for hours
- Protein quantification in solution over 7 logs of linearity (<10 fg to >100 ng of a 30 kDa protein)

5. Quantification of HiBiT-Tagged Proteins on Blots



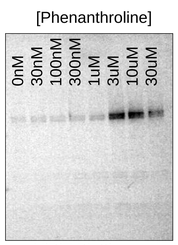


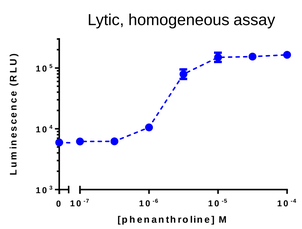
Detection of Purified HaloTag-HiBiT



- Blot added to LgBiT-containing detection reagent
- Detection protocol requires <30 minutes (no blocking, washing or antibody addition steps)
- Dynamic range of >6 logs (100 fg to >100 ng, not in the same exposure)

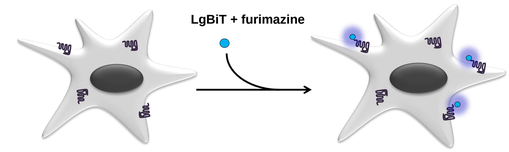
6. Quantification of Regulated Expression of HiBiT-Tagged Proteins

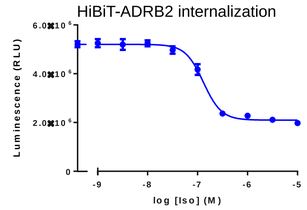




- Changes in the amount of HiBiT-tagged protein caused by altered transcription, translation, or stability can be quantified at endogenous levels using either the lytic or blotting assays.
- Regulated degradation of the transcription factor Hif1a is inhibited by phenanthroline, leading to protein accumulation. HEK293 cells transiently expressing Hif1a-HiBiT were treated with phenanthroline for 4 hours in 96-well format.
- The response was measured both with a homogeneous, lytic assay format (right) and by HiBiT blotting (left) after SDS-PAGE.

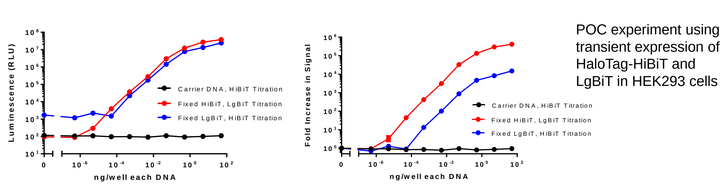
7. Quantification of Extracellular HiBiT with Live Cells





- Quantify cell surface or secreted protein by adding LgBiT-containing detection reagent.
- LgBiT protein is membrane impermeable.
- Live cell, non-lytic assay for receptor internalization, protein trafficking, or secretion.

8. Quantification of Intracellular HiBiT with Live Cells



POC experiment using transient expression of HaloTag-HiBiT and LgBiT in HEK293 cells

- Quantify expression or delivery of BiT #1 in target cells expressing BiT #2 by adding the cell-permeable furimazine substrate in a non-lytic assay.
- Potential applications:
  - Viral infection or replication assays (HiBiT, viral genome; LgBiT, cells).
  - Cell fusion assays (cell type #1, HiBiT; cell type #2, LgBiT)
  - Exosome delivery (e.g., exosome, HiBiT; cells, LgBiT)
  - Live-cell quantification of a HiBiT-tagged protein in a cell expressing excess LgBiT.

9. Conclusions

HiBiT: 11-amino acid protein tag for protein quantification
- A peptide tag that acts like a super-bright luciferase
- Small size reduces any potential impact on fusion partner function

Lytic assay (Nano-Glo® HiBiT Lytic Assay System):
- Quantify expression of HiBiT-tagged proteins with >7-log linear dynamic range
- Femtogram sensitivity
- Simple add-mix-read assay protocol (homogeneous)
- Monitor regulated changes in protein stability

Blotting assay (Nano®-Glo HiBiT Blotting System):
- Determine protein size and quantify expression on blots
- Protocol requires only minutes, not several hours like immunodetection
- Femtogram sensitivity

Extracellular detection (Nano®-Glo HiBiT Extracellular Assay System):
- Quantify changes in surface expression or secretion of HiBiT-Tagged proteins
- Simple add-mix-read assay format that can be performed in minutes

Monitor cell fusion or viral replication in live cells by co-localization of HiBiT and LgBiT

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