HiBiT Protein Tagging System to Study Protein Interactions at Endogenous Levels

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1. Introduction

HiBiT is an 11-amino-acid peptide tag that can be quantified *in vitro* or in live cells through enzyme complementation using sensitive add-and-read bioluminescent assays. The sensitivity of bioluminescence enables use of HiBiT-tagged proteins expressed at endogenous levels. Here we demonstrate the utility of HiBiT-tagging for endogenous interactome mapping using two complementary approaches.

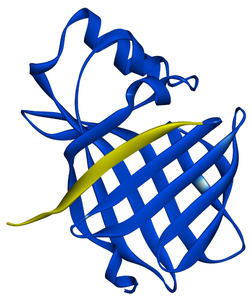
First, we demonstrate a classic immunoprecipitation method with a high-affinity, Anti-HiBiT monoclonal antibody immobilized on magnetic beads.

Next, we demonstrate a proximity-based method called HiBiT-BioID, in which LgBiT is fused to a promiscuous biotin ligase. Upon transfection, LgBiT targets the biotin ligase to the HiBiT-tagged protein, enabling proximity-based biotinylation and the subsequent capture of the biotinylated interactome with immobilized streptavidin. The efficacy of these methods will be discussed in the context of multiple model systems at a variety of expression levels.

2. HiBiT Reporter for Endogenous Protein Tagging

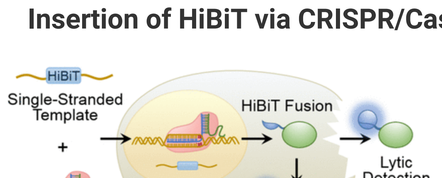
LgBiT
(18 kDa)

HiBiT
(1.3 kDa, 11 a.a.)



- High affinity interaction (KD = 0.7 nM)
- HiBiT/LgBiT complementation produces a bright bioluminescent signal
- HiBiT is ideal for endogenous tagging with CRISPR/Cas9

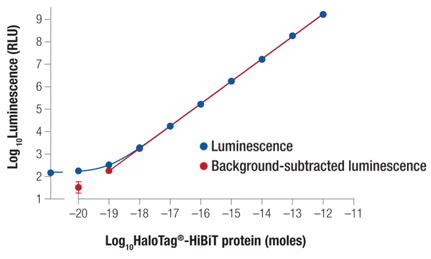
Insertion of HiBiT via CRISPR/Cas9



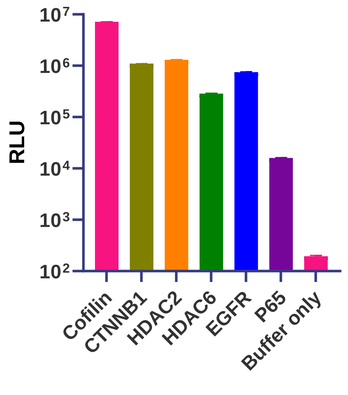
- Small size allows use of DNA oligo template for higher efficiency knock-in.
- Luminescent assays with LgBiT allow easy screening of edited clones.

3. Bioluminescent HiBiT Lytic Assay for Fast and Easy Protein Quantitation

HiBiT Lytic Assay Standard Curve



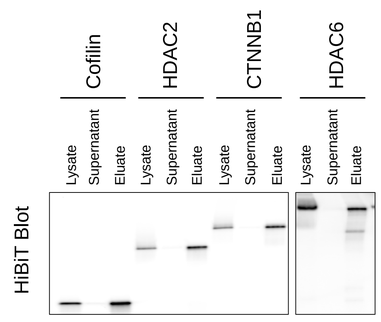
HiBiT Lytic Assay
Quantification of POI-HiBiT in Lysates



RLU → Protein Concentration
(via HiBiT standard curve)

4. Anti-HiBiT Antibody for Affinity Capture of HiBiT-Tagged proteins

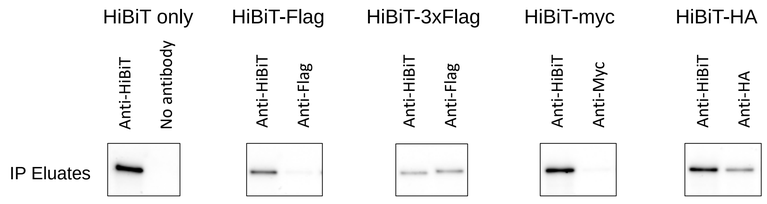
A) Immunoprecipitation of HiBiT-Tagged Proteins from CRISPR Cell Lines



Excellent capture and enrichment of various HiBiT-Tagged proteins.

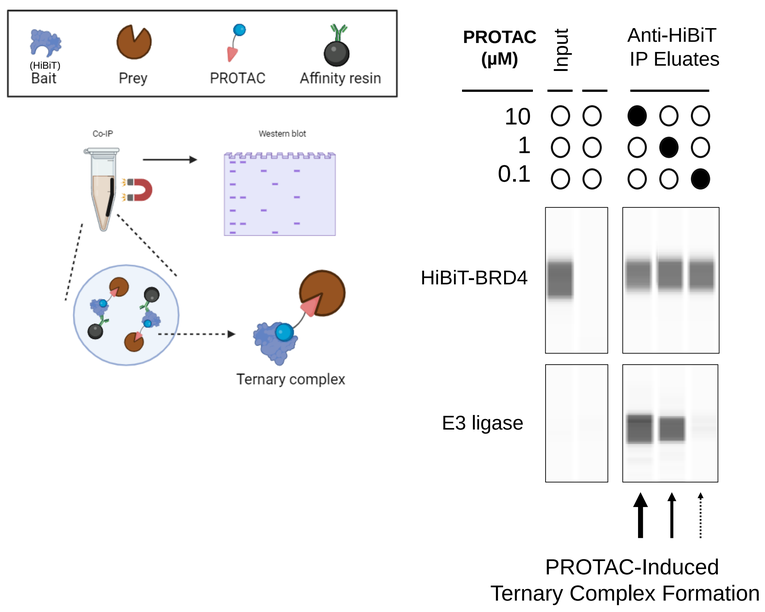
B) Comparison to Immunoprecipitation with other common tags.

- HSP90B1 was tandem-tagged at the C-terminus with HiBiT and HA, Myc, or 1 or 3 copies of FLAG tag using CRISPR/Cas9.



HiBiT performed similarly to 3xFlag and better than all single copy tags.

5. HiBiT Capture for Targeted Protein Degradation

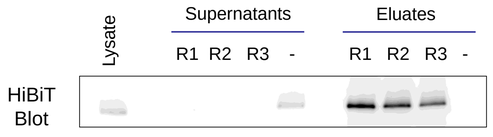


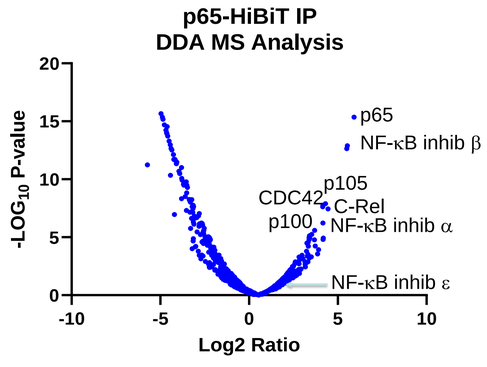
PROTAC-Induced
Ternary Complex Formation

Anti-HiBiT beads successfully co-immunoprecipitate HiBiT-BRD4 and E3 Ligase after PROTAC treatment.

6. p65-HiBiT Co-Immunoprecipitation

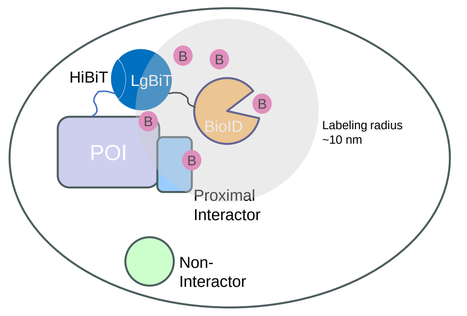
- Anti-HiBiT IP with p65-HiBiT Hela CRISPR knock-in cells and Hela parental cells
- IPs were performed in triplicate and eluates were analyzed by DDA MS





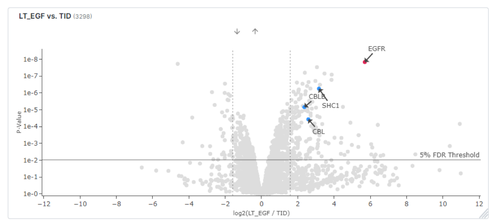
7. HiBiT-BioID Proximity Labeling Concept

HiBiT-BioID: HiBiT-POI + LgBiT-Biotin Ligase Fusion



1. CRISPR HiBiT-POI Cell Line
2. Transfect LgBiT-BioID Fusion (or BioID alone as a spatial control)
3. LgBiT-BioID is targeted to HiBiT
4. Add Biotin to start labeling (B)
5. Lyse and capture biotinylated proteins with streptavidin beads
6. Digest, then LC-MS/MS
7. Bioinformatic Analysis

EGFR Model System:

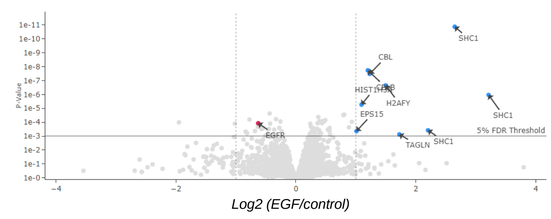


- EGFR-HiBiT HeLa cells were transfected with BioID or LgBiT-BioID, treated with Biotin for 30 minutes, then lysed. Biotinylated proteins were captured on magnetic streptavidin. Three biological replicates were collected in DIA mode. Data were searched with Spectronaut and visualized in Mass Dynamics.

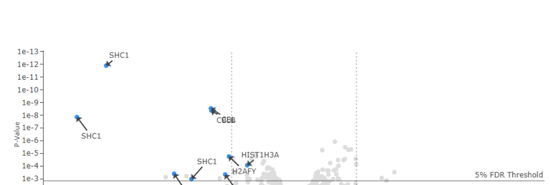
HiBiT-BioID successfully identifies EGFR and known interactors.

8. EGFR-HiBiT-BioID Interactome Modulated by EGF and/or Afatinib

EGF treatment induces changes in the EGFR Interactome



Pre-treatment with afatinib prevents EGF-Induced changes



HiBiT-BioID successfully identifies compound-induced changes in the interactome of EGFR.

9. Conclusions

- Pairing HiBiT tag with CRISPR/Cas9 enables quantitative study of proteins expressed at endogenous levels.
- Add-and-read bioluminescent assays allow simple and sensitive quantification of HiBiT-tagged proteins prior to capture and analysis.
- The specific, high-affinity Anti-HiBiT monoclonal antibody provides superior capture of tagged proteins, even at low expression levels.
- HiBiT-BioID, a proximity-based method, is a useful method for investigating how interactomes change in response to compound treatment.
- The combination of orthogonal methods (IP and HiBiT-BioID) for interactome analysis makes HiBiT a uniquely powerful epitope tag for enabling the study of protein interactions at endogenous levels.

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