Illuminate Your Targets: Endogenous HiBiT Knock-in Monoclones—Inventory + Rapid Custom

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Abstract

Background & Significance

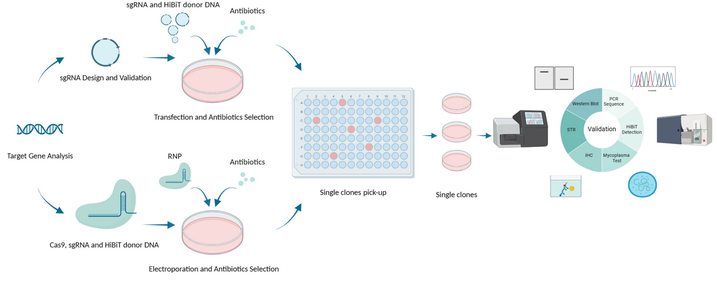
HiBiT is an 11–amino acid tag that complements LgBiT to reconstitute NanoLuc, producing a bright and highly linear bioluminescent signal proportional to endogenous protein abundance. Compared with Western blot–dependent workflows, endogenous HiBiT knock-ins enable real-time, quantitative monitoring of protein dynamics under native regulatory control. These models accelerate mechanistic validation, potency ranking, SAR development, and combination strategy design across diverse modalities, including PROTACs, molecular glues, LYTACs, and AbTACs.

Kyinno HiBiT Knock-In Portfolio

Kyinno has developed a broad, ready-to-use inventory of endogenous HiBiT knock-in monoclonal lines spanning more than 30 genes across key biological categories—oncogenic signaling, kinases, transcription factors, and E3-amenable targets. Representative nodes include KRAS, CDKs, PRMT5, EGFR, AR, BRD family proteins, EZH2, and STATs.This portfolio provides comprehensive coverage of target classes relevant to protein degradation, signal transduction, and transcriptional regulation. Rapid custom knock-in generation (N- or C-terminal) further extends the platform to project-specific targets and variants, enabling teams to begin immediately with inventory models or quickly obtain bespoke constructs.

Methods & Platform Deployment

The system integrates CRISPR–Cas9 HDR–mediated HiBiT insertion at endogenous loci, preserving gene dosage and protein function. Standardized NanoLuc assay formats—including 10-point curves and 48–72-hour or live-cell kinetic measurements—yield quantitative, plate-comparable degradation and turnover profiles.



1 week | 1 week | 3-4 weeks | 3-4 weeks | 2 weeks

List of Cell Lines & Characterization of HiBiT-KI genes

Cell ID	Cell name	Cell ID	Cell name
KC-2151	293T-LDHA-HiBiT-GPRC5D-KI	KC-5555	Calu6-KRT80-HiBiT-KI
KC-4312	293T-GSPT1-HiBiT-KI	KC-5663	NCI-H1975-EGFR-T790M-L858R-HiBiT-KI
KC-4700	TMD8-BTK-HiBiT-KI	KC-5715	22Rv1-ARV7-HiBiT-KI
KC-4812	Jurkat-VAV1-HiBiT-KI	KC-5770	Jurkat-CBL-B-HiBiT-KI
KC-5252	Lncap-AR-HiBiT-KI	KC-5801	K562-IKZF4-HiBiT-KI
KC-5411	Jurkat-STAT6-HiBiT-KI	KC-5814	Calu6-SMARCA2-KO-KRT80-HIBIT-KI
KC-5418	293T-KRAS-HiBiT-KI	KC-6267	SK-N-DZ-SALL4-N-HiBiT-KI
KC-5553	A549-KRT80-HiBiT-KI	KC-6381	293T-CDO1-HiBiT-KI

……(Not an exhaustive list—contact us for details)

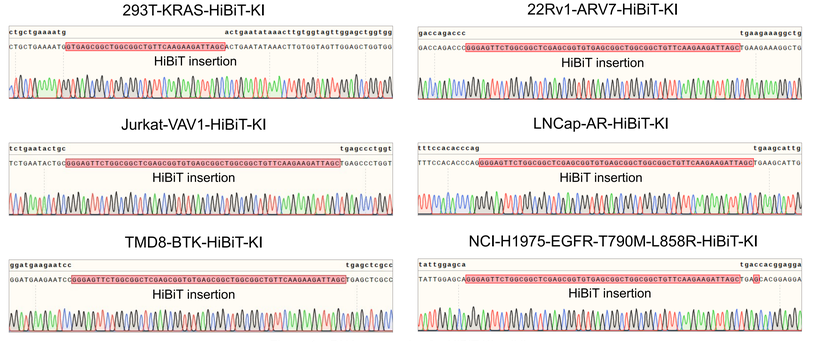


Figure 1: cDNA sequencing for HiBiT-KI cell lines

Characterization of HiBiT-KI proteins

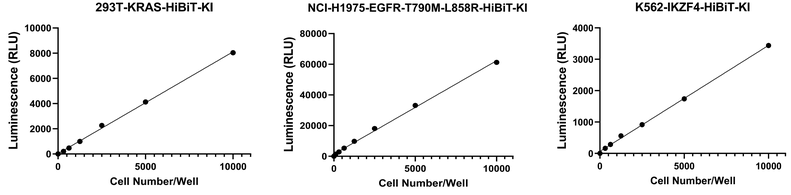


Figure 2: HiBiT detection for 293T-KRAS-HiBiT-KI, NCI-H1975-EGFR-T790M-L858R-HiBiT-KI and K562-IKZF4-HiBiT-KI cell lines

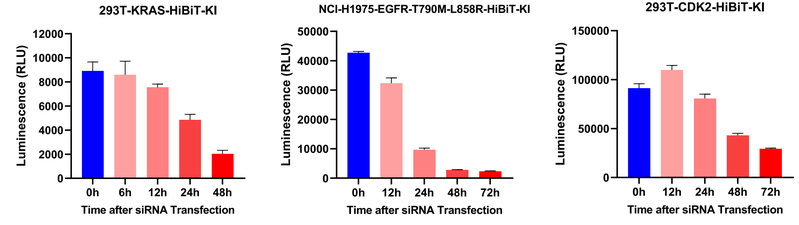


Figure 3: Monitor the degradation process of several proteins via HiBiT

Drug screening using HiBiT-KI cell lines

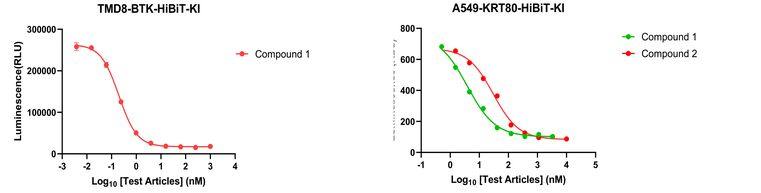


Figure 4: PROTACs screening using TMD8-BTK-HiBiT-KI and A549-KRT80-HiBiT-KI cell lines

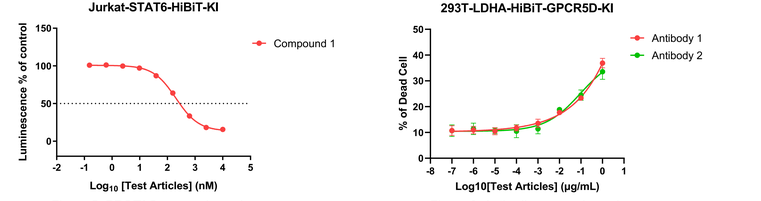


Figure 5: PROTACs screening using Jurkat-STAT6-HiBiT-KI cell line

Figure 6: Antbodies screening using 293T-LDHA-HiBiT-GPRC5D-KI cell line

Conclusion

A panel of cell lines with endogenously HiBiT-tagged proteins was successfully generated via CRISPR technology, enabling sensitive detection of target protein abundance changes.

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