EARLY ACCESS PROTOCOL

Monitoring HiBiT-KRAS(WT) Levels in HiBiT-KRAS(WT) KI HEK293 Cells (CPM)

Instructions for Use of
CS3023186R

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1. Description

This early access material is designed for monitoring HiBiT-KRAS(WT) levels in HEK293 cells that have been genetically modified via CRISPR-Cas9 to fuse HiBiT to the N terminus of KRAS(WT). The resultant clone cells have been verified to be heterozygous for the HiBiT insertion.

2. Product Components and Storage Conditions

PRODUCT	SIZE	PART NUMBER
HiBiT-KRAS(WT) KI HEK293 Cells (CPM)	**1 each**	**CS3023186R**

Includes:

- 2 vials HiBiT-KRAS(WT) KI HEK293 Cells (CPM) (CS3023185)

Storage Conditions: Store the HiBiT-KRAS(WT) KI HEK293 Cells (CPM)[a–e] at –140°C in the liquid nitrogen vapor phase, upon receipt.

3. Before You Begin

Materials to Be Supplied By the User

- white, 96-well plate (e.g., Costar Cat.# 3917)
- miscellaneous tissue culture reagents and equipment
- DPBS (1X) (e.g., GIBCO® Cat.# 14190-144)
- 0.25% Trypsin-EDTA (GIBCO® Cat.# 25200-056)
- CellTiter-Glo® 2.0 Cell Viability Assay (Cat.# G9241)
- Nano-Glo® HiBiT Lytic Detection System (Cat.# N3030, N3040, N3050), which includes: Nano-Glo® HiBiT Lytic Substrate; Nano-Glo® HiBiT Lytic Buffer; and LgBiT Protein
- DMEM (e.g., Thermo Fisher Scientific Cat.# 11995065)
- FBS (e.g., VWR Cat.# 89510-194)
- DMSO (e.g., Sigma Cat.# D2650)

3.A. Media for Cell Propagation and Freezing

cell propagation and assay medium

90% DMEM
10% FBS

freezing medium

85% DMEM
10% FBS
5% DMSO

3.B. Cell Propagation

Thawing Cells for Culture

1. Remove vial from long-term storage (–140°C or below) and incubate in 37°C water bath for 2–3 minutes until thawed.
2. Transfer cells (1ml) into a 15ml centrifuge tube containing 9ml of propagation medium prewarmed to 37°C.
3. Centrifuge for 5 minutes at 200 × *g*.
4. Remove medium and suspend pellet in 10ml of prewarmed propagation medium. Place cell suspension in a T75 tissue culture flask.
5. Culture in a humidified 37°C, 5% CO_2 incubator.
6. Maintain cells in propagation medium and passage when approximately 80% confluent. A subcultivation ratio of 1:3–1:10 is recommended.

Freezing/Banking Cells

7. Remove propagation medium and wash cells once with DPBS.
8. Remove DPBS and add an appropriate volume of 0.25% Trypsin-EDTA, such that the entire surface area of the tissue culture dish is covered.
9. Incubate at 37°C for 5 minutes or until cells can be easily removed from the surface of the tissue culture plate.
10. Add an equal volume of propagation medium to the tissue culture plate and transfer the cell suspension into a conical tube for centrifugation.
11. Centrifuge cells 5 minutes at 200 × *g* and carefully remove supernatant.
12. Gently suspend cell pellet in cold freezing medium at 2×10^6–5×10^6 cells/ml and aliquot into appropriately-sized cryovials.
13. Freeze in a controlled-rate freezer.
14. Store long term in liquid nitrogen vapor phase.

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4. Nano-Glo® HiBiT Lytic Detection Assay Protocol

See *Nano-Glo® HiBiT Lytic Detection System Technical Manual* #TM516 and *CellTiter-Glo® 2.0 Assay Technical Manual* #TM403 for detailed instructions.

4.A. Preparing Cells for Nano-Glo® HiBiT Lytic Assay

1. Adjust the cell density to 2×10^5 cells/ml in assay medium. The cell number can be adjusted to increase luminescent signal.
2. Dispense 100µl per well of cell suspension (2×10^4 cells) into a white 96-well plate.
3. Plate replicates for both the lytic assay and the cell viability assay at the same time. Do not place the lytic and viability samples in adjacent wells, as the luminescence from the CellTiter-Glo® 2.0 Reagent may be bright enough to affect readings in neighboring wells. If placing lytic and cell viability samples in adjacent wells is unavoidable, perform the Nano-Glo® HiBiT Assay first, read the luminescence, then perform the CellTiter-Glo® Assay.
4. Cells can be assayed the day of plating or later, depending on the type of plate and cell density.

4.B. Nano-Glo® HiBiT Lytic Detection Assay

Immediately prior to Nano-Glo® HiBiT Lytic measurements, prepare 2X Nano-Glo® HiBiT Lytic Detection Reagent by diluting LgBiT Protein 1:100 and Nano-Glo® HiBiT Lytic Substrate 1:50 into an appropriate volume of room temperature Nano-Glo® HiBiT Lytic Buffer. Prepare enough 2X detection reagent for the number of wells to be assayed, including extra volume to account for pipetting error (for example, number of wells + 10%).

1. Add 100µl per well of 2X Nano-Glo® HiBiT Lytic Detection Reagent for a 96-well plate. Mix plate on an orbital shaker for 10–20 minutes at 500rpm.
2. Measure luminescence on a GloMax® Discover System (Cat.# GM3000) or other plate-reading luminometer.

4.C. CellTiter-Glo® 2.0 Cell Viability Assay

1. Equilibrate CellTiter-Glo® 2.0 Reagent to room temperature prior to starting the assay.
2. During the Nano-Glo® HiBiT Lytic Assay, plate replicate wells to examine cell viability with a final volume of 100µl per well.
3. Add 100µl per well of CellTiter-Glo® 2.0 Reagent for a 96-well plate. Mix plate on an orbital shaker for 10–20 seconds at 350rpm.
4. Measure luminescence on a GloMax® Discover System or other luminometer.

4.D. Calculating Signal-to-Background Ratios

$$\text{Signal-to-background ratio} = \frac{\text{S/CTG, S}}{\text{P/CTG, P}}$$

where:

S = sample reporter signal

P = parental/control cells reporter signal

CTG, S = sample CellTiter-Glo® 2.0 signal

CTG, P = parental/control cells CellTiter-Glo® 2.0 signal

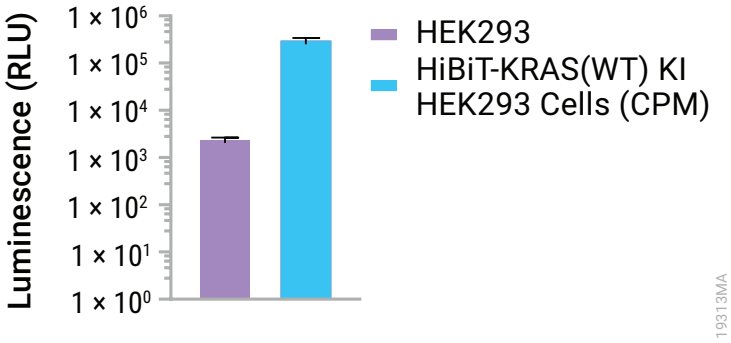

Figure 1. Measurement of endogenously-tagged HiBiT-KRAS(WT) by the Nano-Glo® HiBiT Lytic Detection Assay. HEK293 parental cells and HiBiT-KRAS(WT) KI HEK293 Cells (CPM) were plated into a white 96-well assay plate at 20,000 cells/well in triplicate. HiBiT-KRAS(WT) levels were measured by adding an equal volume of 2X Nano-Glo® Lytic Assay Detection Reagent. Luminescence was measured using a GloMax® Discover System. Relative light units (RLU) are shown on the y axis. Normalizing to CellTiter-Glo®, the signal-to-background ratio is approximately 156.6 after thawing (data not shown). Data were graphed and analyzed using GraphPad Prism® software.

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