NanoBRET Implementation in HTS For Drug Discovery

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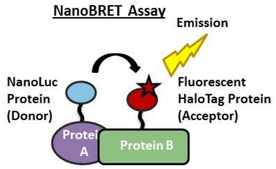
Abstract # 5017

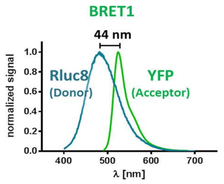
1. Introduction

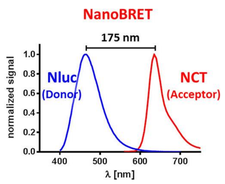
Identifying small molecule modulators, inhibitors or activators, of protein-protein interactions (PPI) remains challenging, largely due to the difficulty of developing robust and high-throughput screening tools. Bioluminescent resonance energy transfer (BRET) has been used to monitor real-time protein:protein interactions in cells but current approaches suffer from limited sensitivity and narrow dynamic range. Here we present a new BRET method, termed NanoBRET, based on a small and extremely bright NanoLuc luciferase coupled to a HaloTag - long-wavelength fluorophore. This highly effective energy donor-acceptor combination boosts BRET performance and the higher sensitivity facilitates application of the method in high density plates and high throughput screening (HTS). Application of acoustic dispensing instruments simplifies assay assembly in HTS formats by allowing dispensing of stock reagents without pre-dilutions. We present examples of inhibitor screening for bromodomain/histone and p53/Mdm2 transcriptional protein interactions; as well pathway activators for the EFGR/GRB2 membrane interaction and cRaf/bRaf signaling pathway.

2. NanoBRET Principles and Advantages

(Nano)**B**ioluminescent **R**esonance **E**nergy **T**ransfer

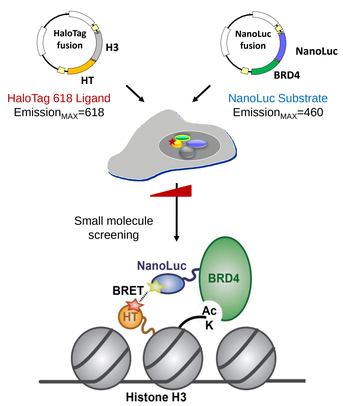






- Energy transfer from bioluminescent donor to fluorescent acceptor resulting in emission of light.
- Allows measurement of protein interactions (PPI) with binding partners in cells.
- New Nano-BRET assays substantially increase light output and optimized spectral separation increases signal/noise ratio.

3. NanoBRET PPI and Small Molecule Screening Workflow



Workflow example for BRD4/ Histone H3 Assay

1. Bulk co-transfection of donor and acceptor.
2. Re-plating, +/- addition of HaloTag 618 ligand, and addition of small molecule* being screened followed by 4-24 hour treatment.
3. Addition of NanoLuc substrate (by acoustic dispensing), measurement of signal, and calculation of BRET ratio.

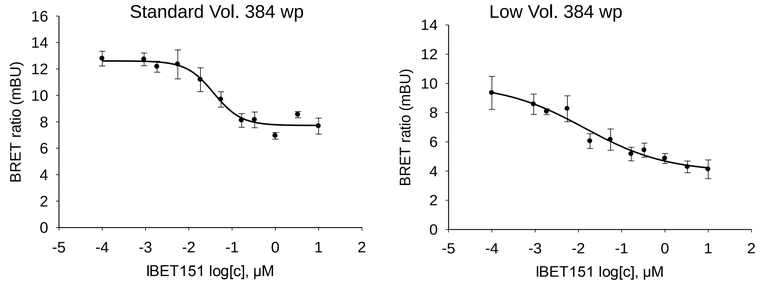
4. Nanoliter Acoustic Dispenser ATS from EDC Biosystems

Acoustic dispensing uses sound waves focused on the surface of a liquid to generate precise and accurate droplets in the range of 1nl to 25nl volumes. Dispensing of multiple droplets enables a dispensing range of 1nl to 1µl. Droplets are directed into an inverted well plate where they adhere to the bottom of a well via surface tension.

Acoustic Dispensing Advantages

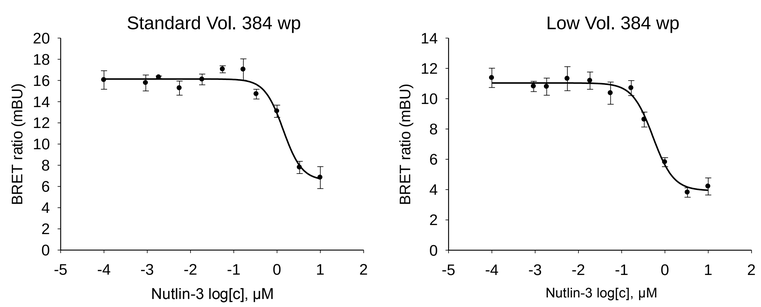
- Accurate and precise nanoliter transfers
- Low dead volumes
- Dispense range from 1nl to 1µL
- Variable droplet size allows optimization for dispense speed or volume resolution
- Easy to automate
- Lasting precision and performance
- Open platform for application flexibility
- Wide variety of plates

5. Interaction of BRD4/H3 is Inhibited by IBET151



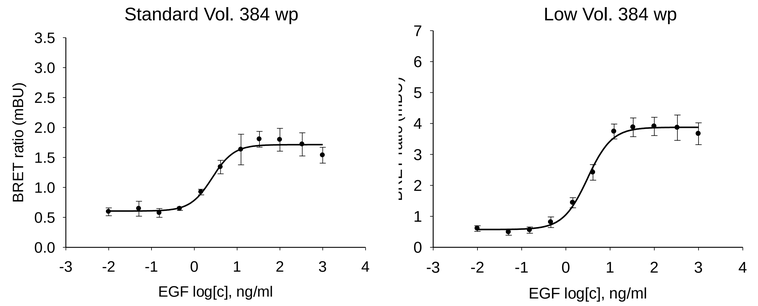
- IBET151 inhibits the interaction with expected IC_{50}=37 nM values obtained in standard and low volume 384 well plates
- Nanoliter dispense of IBET151 inhibitor from stock and pre-diluted solution with ATS

6. Inhibition of p53/Mdm2 by Nutlin-3



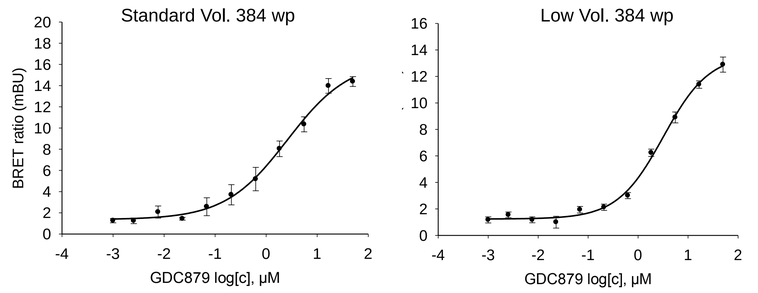
- Nutlin-3 inhibits the interaction with expected IC_{50} =1.3 µM values obtained in standard and low volume 384 well plates
- Nanoliter dispense of Nutlin-3 inhibitor from stock and pre-diluted solution with ATS

7. Stimulation of EGFR/GBR2 Interaction by EGF



- EGF stimulates the interaction of membrane protein EGFR and its adaptor protein GRB2 with IC_{50}=2.6 ng/ml values obtained in regular and low volume 384 well plates
- Nanoliter dispensing of EGF from water stock and pre-diluted solutions with ATS

8. cRaf/bRaf Dimerization by GDC0879 Compound



- GDC0879 promotes the dimerization of cRaf/bRaf in the EGFR pathway with IC_{50}=3.2µM values obtained in regular and low volume 384-well plates
- Nanoliter dispensing of GDC0879 from DMSO stock and pre-diluted solutions with ATS

9. Conclusions

New BRET technology offers increased detection sensitivity and dynamic range

- Enabled by significantly greater “donor” light intensity and
- Optimized spectral resolution (175nm) between “donor” and “acceptor”

NanoBRET PPI screening benefits

- Specific, robust, and sensitive
- Live cell in real-time with kinetic or end point measurements
- Ratiometric assay - insulates against variations in cell number/expression, etc.
- Simple measurement – ratio with or without background subtraction
- Bioluminescent eliminates high background, photobleaching and autofluorescence

Dispensing by ATS instrument facilitates HTS format

- Dose response curves of compounds and NanoLuc substrate can be added directly without pre-dilutions
- Compatible with standard and low volume 384 well formats for assay miniaturization

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