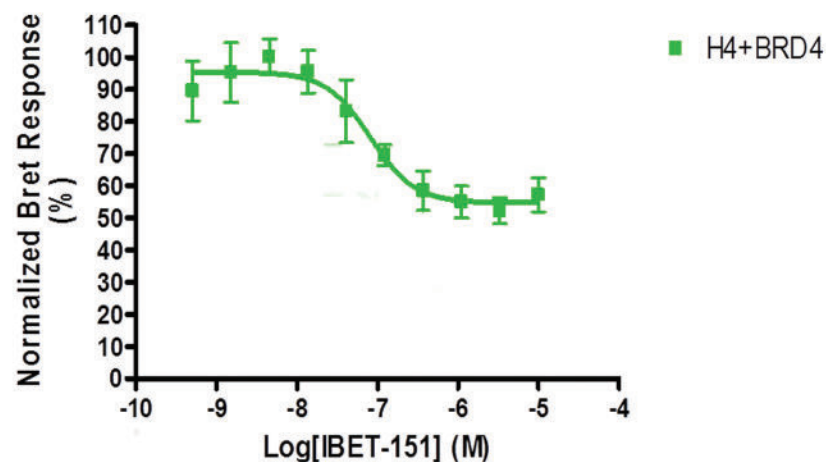


## Monitoring Epigenetic Protein and Protein Interactions in Living Cells Using NanoBRET Technology

**HEK-293 Cells:** Human embryonic kidney cell line from ATCC.

**Assay format:** NanoBRET assay.

**Assay Protocol:** HEK-293 cells expressing H4-HaloTag and Nanoluc-BRD4 were plated in 384-well format and treated with various concentrations of BRD4 inhibitor IBET-151 for 22 hours before addition of Nano-Glo Luciferase Assay Substrate and NanoBRET measurement on an Envision 2104 Multilabel Reader equipped with 460/25BP and 600/LP filters. The IC<sub>50</sub> (EC<sub>50</sub>) value was calculated and IC<sub>50</sub> curve was plotted using the GraphPad Prism 4 program to show the dose response effect of IBET-151 on the interaction of Histone H4 and BRD4 .



|           |            |
|-----------|------------|
|           | H4+BRD4    |
| HILLSLOPE | -1.583     |
| EC50      | 8.180e-008 |