**NanoBRET™ Target Engagement Assay for RIOK2 Kinase**

**Cell line:** HEK293 human epithelial cell line from ATCC.

**Assay format:** 384-well plate format using Promega’s NanoBRET™ TE Intracellular Kinase Assay.

**Assay protocol:** HEK293 cells transiently expressing NanoLuc® RIOK2 Fusion Vector were seeded into the wells of 384-well plates. The cells were pre-treated with the NanoBRET™ Tracer K-5 and then treated with reference compound CTx-0294885 for 1 hour. The BRET signal was measured on an Envision 2104 Multilabel Reader. IC$_{50}$ value was calculated and IC$_{50}$ curve was plotted using the GraphPad Prism 4 program based on a sigmoidal dose response equation.

**Reference compound:** CTx-0294885 (IC$_{50}$ value): 6.913e-007 M

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**Reference Compound IC50 for RIOK2**

<table>
<thead>
<tr>
<th>Normalized Bret Response (%)</th>
<th>-11</th>
<th>-10</th>
<th>-9</th>
<th>-8</th>
<th>-7</th>
<th>-6</th>
<th>-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>120</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>

**Log[CTx-0294885] (M)**

-11 -10 -9 -8 -7 -6 -5

CTx-0294885

**HILLSLOPE** | -1.047
**EC50** | 6.913e-007

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