Activation of the NFκB pathway induces a signal transduction cascade that results in the phosphorylation, ubiquitination, and proteasomal degradation of IκBα. Upon IκBα degradation, liberated NFκB translocates to the nucleus to activate target gene expression (Fig 1). We have developed a set of target-specific HTS-compatible assays capable of interrogating the ubiquitin-proteasome system. Using a clonal cell line expressing GFP-IκBα, we have developed a TR-FRET assay capable of measuring ubiquitination of endogenously expressed IκBα. We have concurrently developed a living-cell β-lactamase (bla) reporter assay for degradation of Bla-IκBα fusion proteins. These technologies provide a powerful means to interrogate the intermediate steps in NFκB signaling, without compromising the endogenous physiological complexity of this signaling pathway. Cellular HTS assays that interrogate these processes will provide a unique integrated approach to dissecting the ubiquitin-proteasome system in the context of NFκB signaling.

**Figure 1** - NFκB Pathway

**Figure 2** - Inducible degradation of GFP-IκBα in HEK-293 cells by Western analysis

<table>
<thead>
<tr>
<th>TNFα, ng/ml</th>
<th>85 kDa</th>
<th>60 kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>.03</td>
<td>.08</td>
</tr>
<tr>
<td>.25</td>
<td>2.2</td>
<td>7.6</td>
</tr>
<tr>
<td>2.6</td>
<td>2.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Figure 3** - Inducible degradation of Bla-IκBα in HEK-293 cells

**Figure 4** - Workflow for cellular GFP-IκBα ubiquitination assay

- Treat cells with agonist or antagonist compounds
- Lyse cells with mild detergent
- Add Tb-anti-ubiquitin antibodies
- Read 520nm/495nm time-gated Emission ratio

**Figure 5** - Lanthascreen™ TR-FRET assays for ubiquitination of GFP-IκBα in HEK-293 cells

**Figure 6** - β-Lactamase assays for Bla-IκBα degradation in living HEK-293 cells

**A** Image Analysis of Bla-IκBα degradation

**B** TNFα-induced degradation of Bla-IκBα

**C** Inhibition of degradation of Bla-IκBα