



SIGNALING PATHWAY REPORTER ASSAYS

A Simple, Orthogonal Screening Tool for Understanding Therapeutic MOAs

EVALUATE DOWNSTREAM RECEPTOR RESPONSES FOR COMPREHENSIVE UNDERSTANDING OF YOUR THERAPEUTIC MOLECULE'S MOA

The PathHunter® Signaling Pathway Reporter assay platform is easy-to-use, functional cell-based assays for quantifying the activation and inhibition of various signaling pathways – optimized for screening both small molecules or biologic drugs. These assays provide a downstream (transcription/translational) read-out that is complementary to upstream receptor-based assays to ultimately gain a comprehensive understanding of your drug molecule's MOA targeting TNF α , PD-1, IL-6, CD40, RANK and other receptors.

- **Greater Understanding** – Assess both receptor-upstream and -downstream responses for a comprehensive understanding of the drug molecule's MOA
- **Versatile** – Suitable for screening of agonists or antagonists, and capable to build assays to interrogate other pathways
- **Simple Protocol, Fast Results** – Easy-to-run, rapid homogeneous protocol amenable to implementation in multiple labs and high-throughput format for increased efficiency
- **Biologically-Relevant** – MOA-reflective, functional assays for monitoring diverse signaling and testing of targeted therapeutics

PathHunter SIGNALING PATHWAY REPORTER ASSAY PRINCIPLE

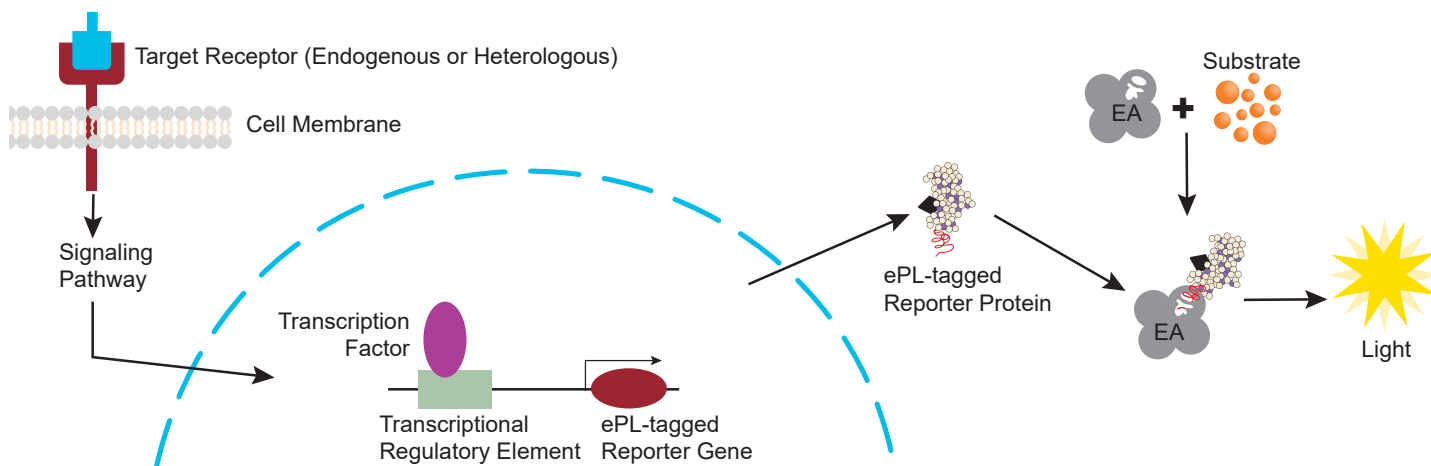


Figure 1. Signaling reporter, cell-based assays involve Enzyme Fragment Complementation detection to monitor the activation or inhibition of various signaling pathways.

Visit discoverx.com/reporters to learn more about these signaling pathway reporter assays.

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QUANTIFY THE ACTIVATION AND INHIBITION OF SIGNALING PATHWAYS

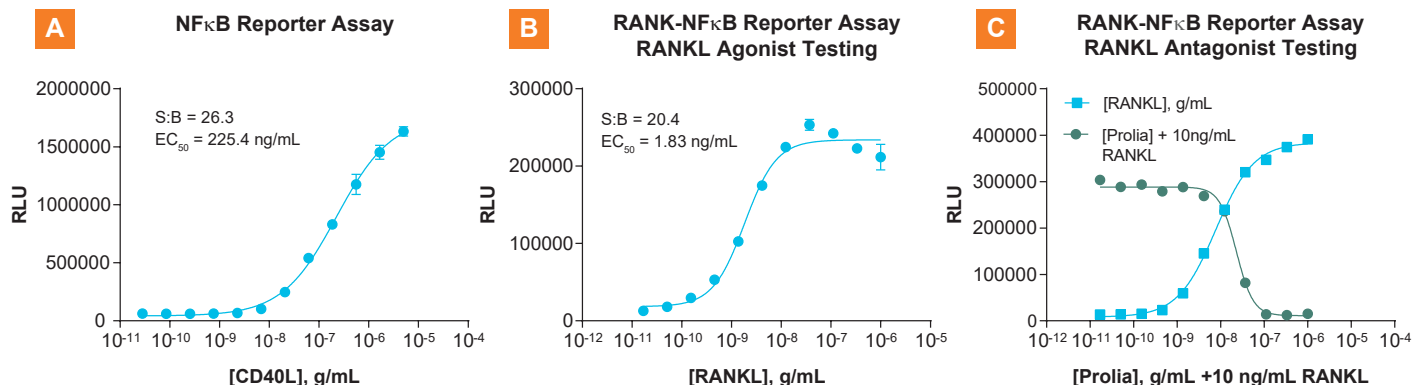


Figure 2. PathHunter® U2OS signaling reporter assays for endogenous or heterologously-expressed target receptors. **A.** NF-κB reporter assay was used to detect CD40L-mediated activation of endogenous CD40 receptors. **B.** RANK-NFκB reporter assay was used to measure RANK activation by RANKL and **C.** analyze antagonist activity of the therapeutic RANKL inhibitor, Prolia® (registered trademark of Amgen).

DEVELOP POTENT AGONIST AND ANTAGONIST THERAPEUTICS

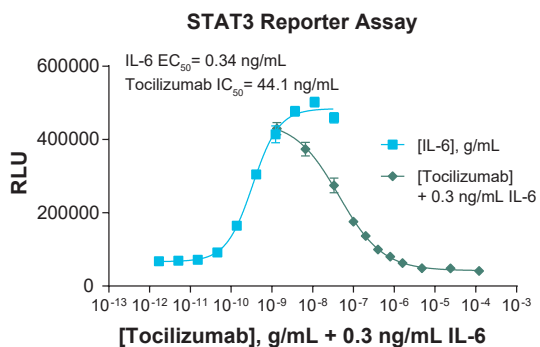


Figure 3. Develop therapeutics targeting receptors with the various signaling pathway reporter assays. The PathHunter HepG2 STAT3 Pathway Reporter Assay was used to detect the potent antagonist therapeutic, Tocilizumab (Actemra®, registered trademark of Genentech), which has been approved for clinical trials to treat patients with severe Covid-19 pneumonia.

MEASURE SENSITIVE RESPONSES, FROM EITHER DOWNSTREAM OR UPSTREAM EVENTS

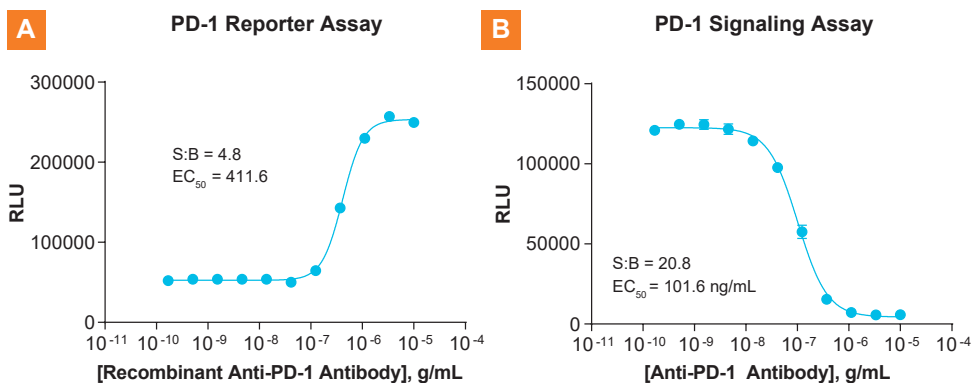


Figure 4. Comparison of antagonist testing using an Anti-PD-1 Antibody results from two different PathHunter Jurkat PD-1 Assays. **A.** The PathHunter PD-1 Pathway Reporter Assay was used to measure downstream effects of PD-1 receptor signaling resulting in NFAT-regulated reporter protein expression. Conversely, **B.** the PathHunter PD-1 SHP2 Signaling Assay was used to measure upstream PD-1 signaling events independent of T cell receptor activation. Both assays are robust and measure inhibition with sensitive responses, from either downstream or upstream events.

Contact us at NA_CAD@discoverx.com to discuss development of additional signaling pathway reporter assays for your drug discovery program.