Immuno-Oncology Therapeutics Development Using PathHunter Signaling Pathway Reporter Assays

eurofins | DiscoverX

The Eurofins Discovery PRODUCTS COMPANY

Jennifer Lin-Jones, Ph.D., Hyna Dotimas, Nishita Matcha Balaguruvappa, Paul Shapiro, Ph.D., Sangeetha Gunturi, Alexander Baumann, Natalie Gath, Ph.D., and Jane Lamerdin, Ph.D.
Eurofins DiscoverX | Fremont, CA 94538

Abstract

Reporter genes are a well-established method used to develop cell-based assays for testing drugs that inhibit or activate targets involved in specific signaling pathways. Here, we introduce PathHunter® Signaling Pathway Reporter Assays that utilize the industry-validated Enzyme Fragment Complementation (EFC) technology to detect reporter gene activity for signaling pathways used by immuno-oncology targets.

In this poster, we show NF-κB and NFAT signaling pathway reporter assays for endogenous or heterologously-expressed target receptors. The NFAT assay is used to measure T-cell activation and has been modified to build assays interrogating PD-1 and TREM1 pathways by the addition of the relevant receptors to the complementary NFAT signaling assay cell line. We also compare the established PD-1 signaling pathway assay with its complementary PD-1 pathway reporter assay. The PD-1 signaling assay captures a proximal signaling event (via SHP-recruitment), while the PD-1 pathway reporter assay quantifies downstream effects of PD-1 (that require T-cell activation). Both assays produce robust and sensitive responses when tested with PD-1 antagonist antibodies.

PathHunter Signaling Pathway Reporter Assays

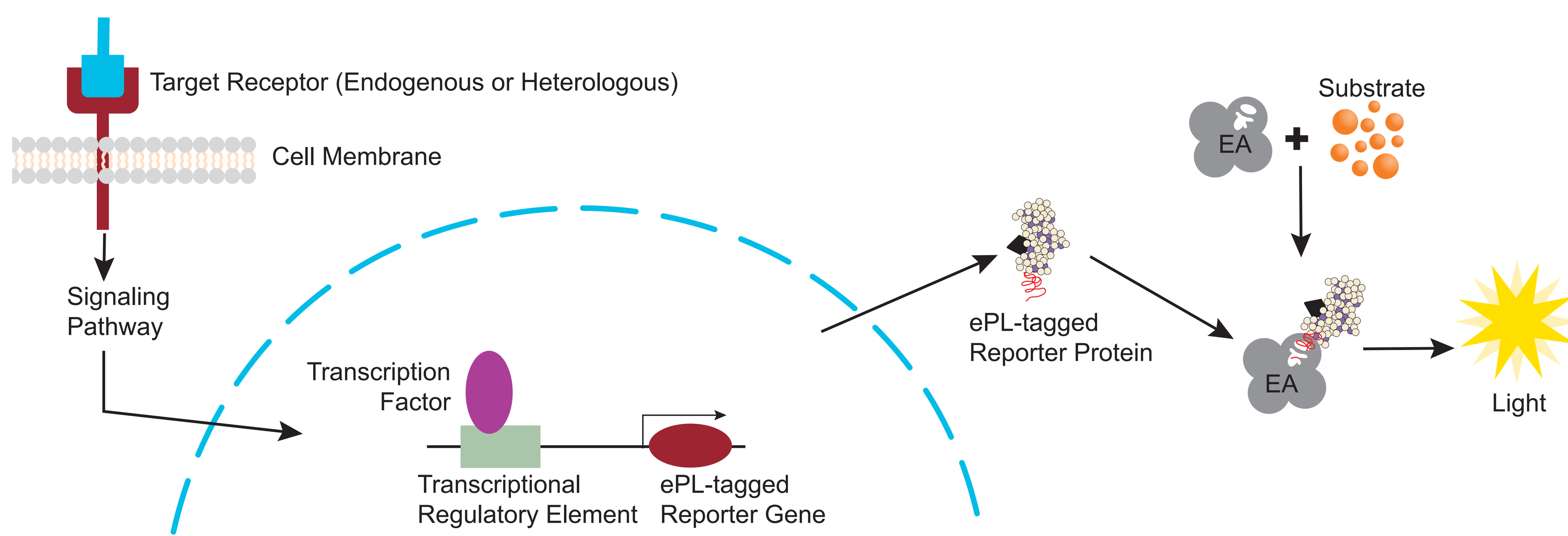

Figure 1. **The PathHunter signaling pathway reporter assay detects target pathway signaling through the activation of endogenous receptors or receptors introduced into cells with the reporter gene construct.** Ligand-mediated stimulation of these receptors initiates pathway signaling and subsequent activation of transcription factors, which bind to a regulatory transcriptional element controlling reporter gene expression. In this assay, the activated signaling pathway drives the expression of the reporter protein tagged with the small enhanced ProLabel (ePL) β-galactosidase enzyme donor fragment. Reporter activity is measured by lysing reporter pathway cells with a detection reagent containing the complementary enzyme acceptor (EA) fragment and luminescent enzyme substrate. The enzyme activity is then detected as a result of EFC.

Cell-Based Signaling Pathway Reporter Assays Using Endogenous or Heterologously-Expressed Target Receptors

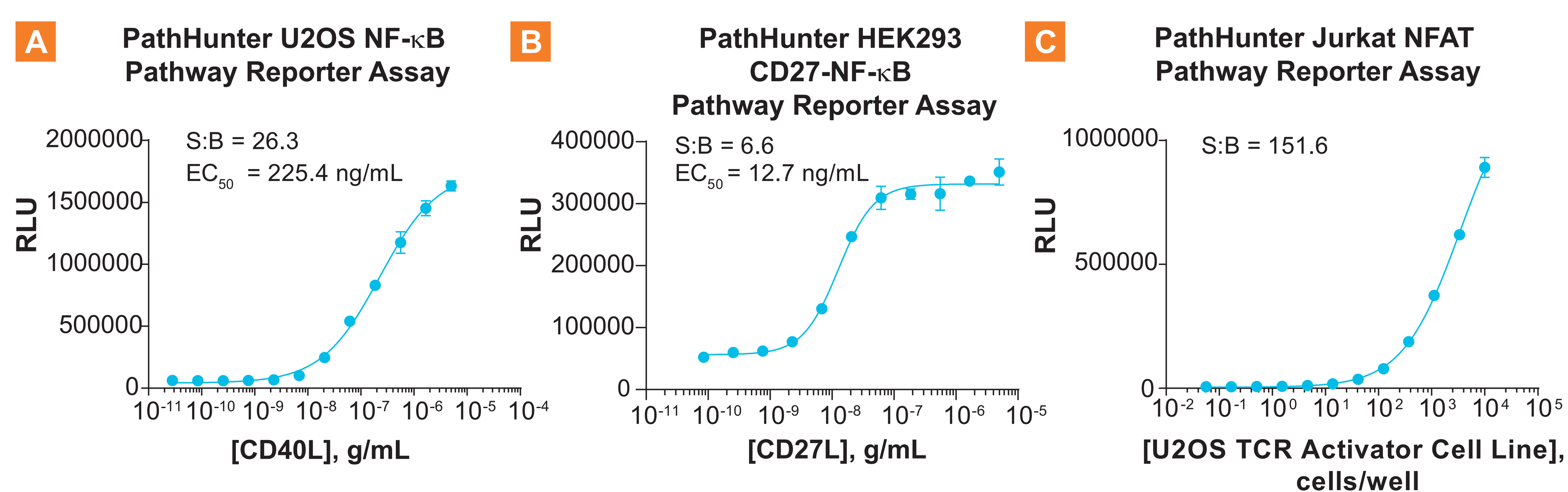

Figure 2. **Pathway signaling reporter assays for endogenous or heterologously-expressed target receptors.** A. The PathHunter U2OS NF-κB Pathway Reporter Assay detects CD40L-mediated activation of endogenous CD40 receptors that signal through the NF-κB pathway. B. The PathHunter HEK293 CD27-NF-κB Pathway Reporter Assay was created by heterologous expression of the co-stimulatory receptor, CD27, in the PathHunter HEK293 NF-κB Pathway Reporter cells, resulting in a sensitive assay to characterize CD27 agonists. Currently, a CD27 agonist antibody, Varlilumab, is in immunotherapy trials. C. The PathHunter Jurkat NFAT Pathway Reporter Assay monitors T-cell activation signaling through T-cell receptors (TCRs) and results in changes in NFAT-regulated reporter gene expression. Jurkat NFAT reporter cells were co-cultured with increasing numbers of U2OS cells expressing a molecule that activates TCRs endogenously expressed in Jurkat cells. When a sufficient number of U2OS T-cell activator cells engage TCRs on the Jurkat cells, TCR signaling results in NFAT-dependent transcription and translation of an ePL-tagged reporter protein.

A PathHunter Jurkat TREM1-DAP12 NFAT Pathway Reporter Assay for Screening Immunomodulators

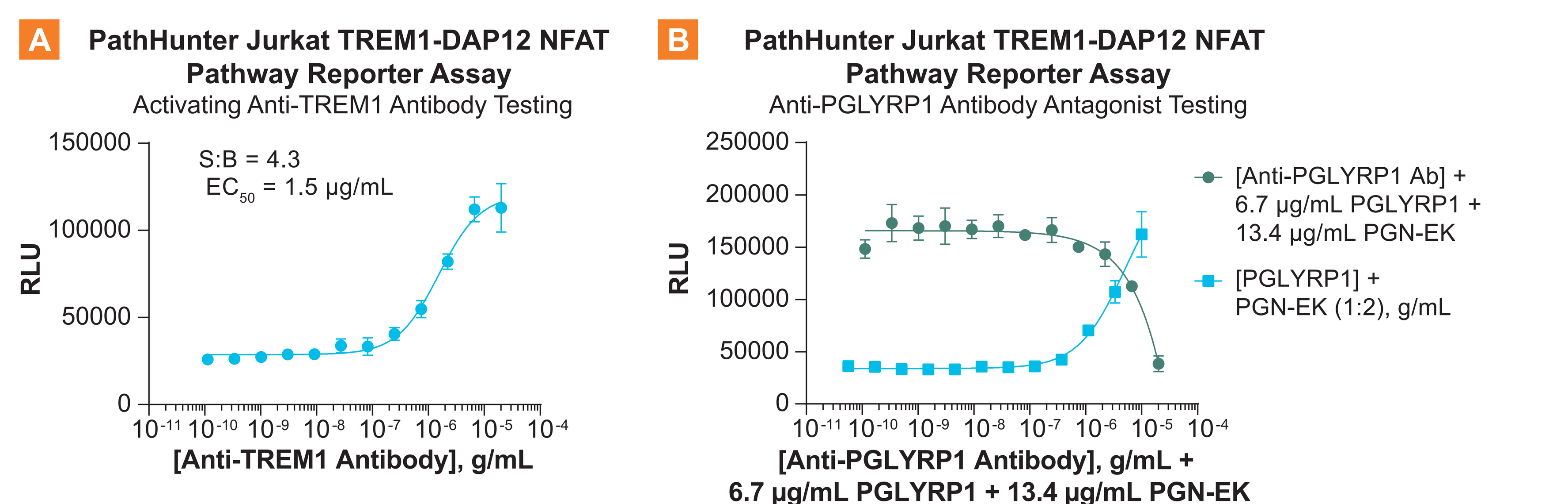

Figure 3. **PathHunter Jurkat TREM1-DAP12 NFAT Pathway Reporter Assay.** TREM1 is highly expressed in myeloid cells and is involved with amplification of inflammatory responses. A role for TREM1 in promoting the tumor microenvironment has also been identified. A. A commercial TREM1 antibody was used to activate TREM1 signaling and increase reporter protein expression. B. The TREM1 ligand PGLYRP1 in combination with PGN-EK was used to activate TREM1, but PGLYRP1 TREM1 activation was inhibited with an Anti-PGLYRP1 antibody. Anti-PGLYRP1 Antibody + 6.7 μg/mL PGLYRP1 + 13.4 μg/mL PGN-EK exhibited a decrease in signal with an S/B ratio of 4.4, while PGLYRP1+PGN-EK in a 1:2 ratio resulted in an increase in signal with an S/B ratio of 4.9.

Signaling Pathway for PD-1 Inhibition of T-Cell Receptor Activation

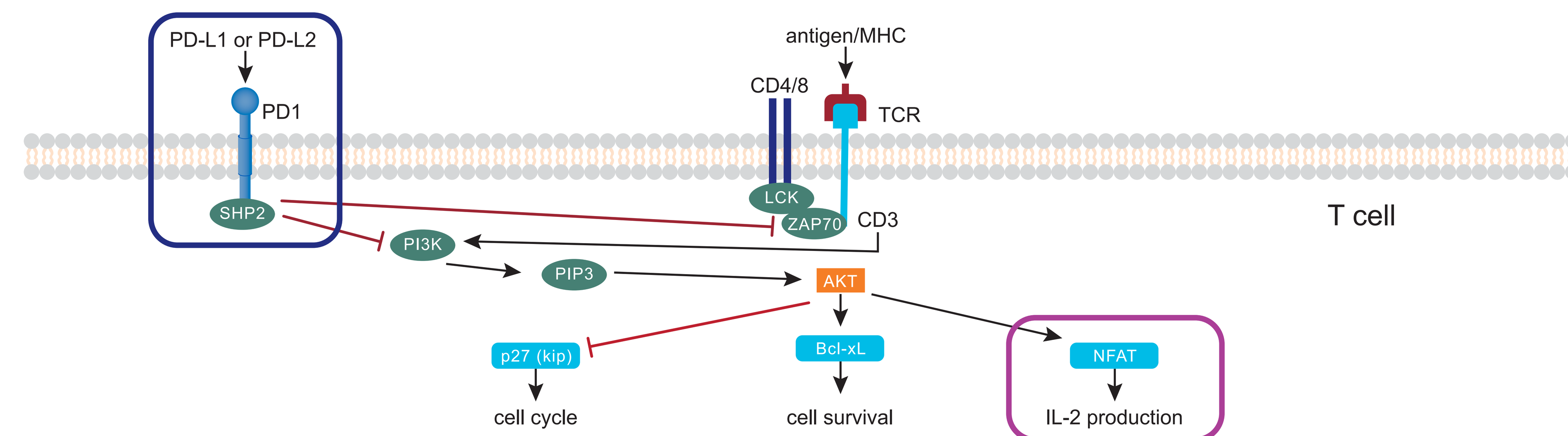

Figure 4. **Graphical depiction of proximal and distal PD-1 signaling events that result in T-cell receptor inhibition.** Upstream events (boxed in blue) are measured with the PathHunter PD-1 Signaling Assay (Figure 6), while the PathHunter PD-1 Pathway Reporter Assay measures NFAT-regulated events that are much further downstream of PD-1 receptor activation (boxed in purple).

Reporter-Based, Inhibitory Checkpoint Receptor Assay Principle

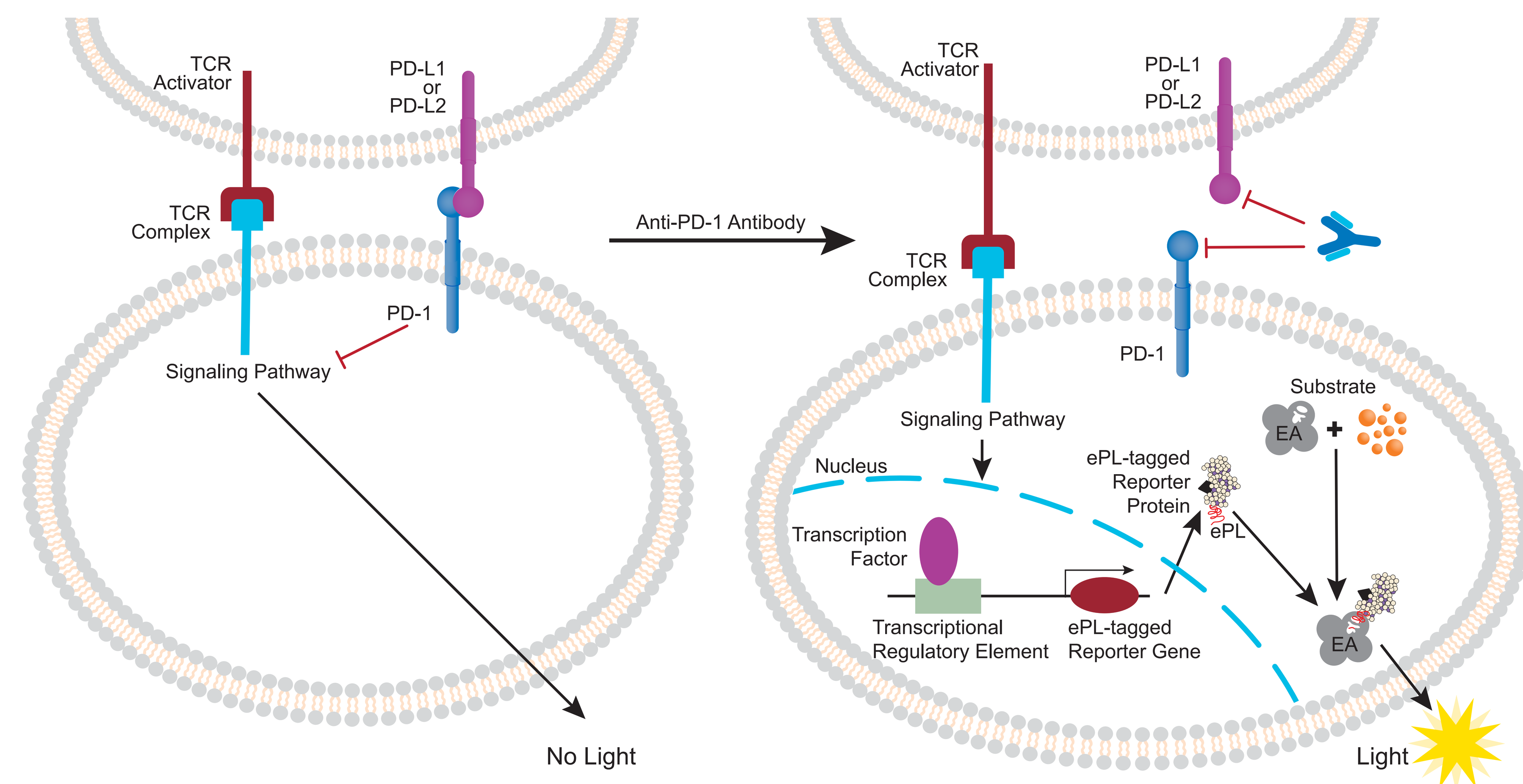

Figure 5. **The PathHunter PD-1 Pathway Reporter Assay Principle.** This assay monitors effects of PD-1 inhibitors by measuring T-cell activation resulting in increased NFAT-dependent expression of a reporter gene tagged with the ePL enzyme donor fragment. PD-1 is heterologously expressed in the PathHunter Jurkat NFAT reporter cell line, and co-cultured with U2OS PD-L1 ligand cells co-expressing a TCR activator, resulting in attenuated TCR activation. Pre-incubation of Jurkat PD-1 cells with an antagonist PD-1 antibody releases PD-1 inhibition of TCR signaling, and NFAT-controlled reporter expression induced by the TCR activator is detected.

Non-Reporter-Based, Inhibitory Checkpoint Signaling Assay Principle

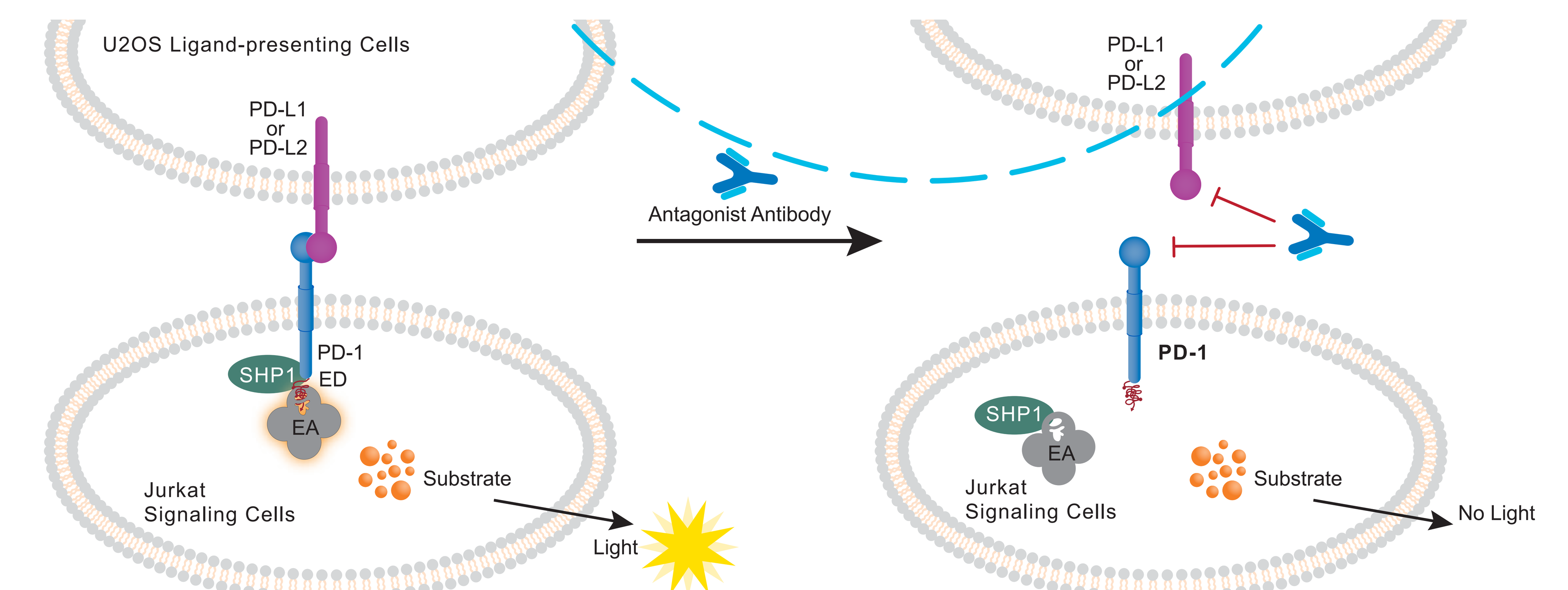

Figure 6. **Assay principle of the non-reporter based PathHunter PD-1 Signaling Assay for comparison with the reporter assay principle (Figure 5).** This PD-1 assay measures SHP1 recruitment to phosphorylated tyrosines found in PD-1 ITIM motifs. When U2OS PD-L1 Ligand Cells are co-cultured with Jurkat PD-1 Signaling Cells, PD-L1 activates the PD-1 receptor and the SHP1 SH2-EA enzyme fragment fusion protein is recruited to PD-1 tagged with the complementary enzyme donor (ED) tag. Antagonist antibody addition disrupts PD-1 interaction with PD-L1, inhibits PD-1 signaling, and results in a loss of chemiluminescent signal.

Complementary PathHunter Assays for PD-1 Pathway Analysis

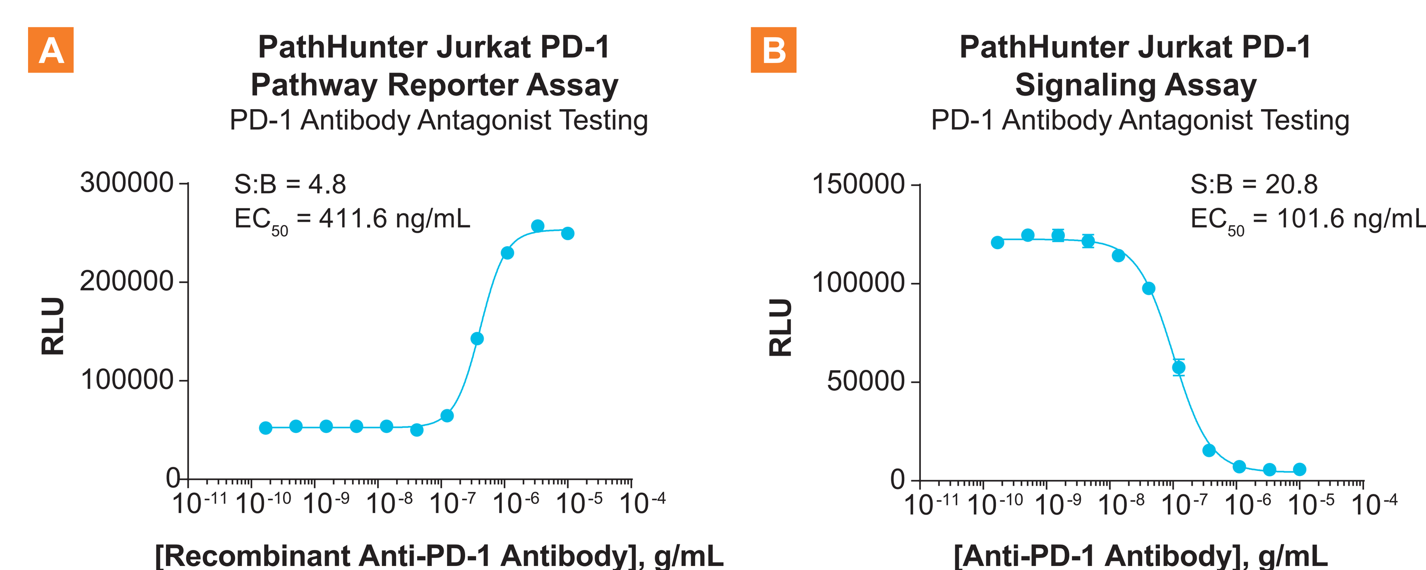

Figure 7. **Comparison of antagonist testing results from the two different PathHunter PD-1 Assays.** A. The PathHunter PD-1 Pathway Reporter Assay was tested with an Anti-PD-1 Antibody. Reporter assay cells co-cultured with the PathHunter U2OS PD-L1/TCR activator cell line results in increased reporter expression due to blocking PD-1 inhibition of TCR activation, resulting in increased NFAT-regulated gene expression, which is measured by this reporter assay. Conversely, B. represents results from the PathHunter Jurkat PD-1 SHP2 Signaling Assay. An Anti-PD-1 Antibody was used to block PD-1 activation mediated by PathHunter U2OS Ligand Cell Line co-culture. This assay measures proximal PD-1 signaling events independent of TCR activation. Both assays are robust and measure inhibition with sensitive responses, from either distal or proximal events. NOTE: The PathHunter Jurkat PD1 Signaling Assay can also be used to develop PD1 agonists.

Summary

- A subset of DiscoverX PathHunter Pathway Reporter Assays relevant for screening or characterization of immuno-oncology therapeutics are presented here. Several of these stable reporter cell lines can be used to generate additional assays for novel immunotherapy targets. For example, the PathHunter Jurkat NFAT Pathway Reporter Assay was used to develop an assay to quantify activity of therapeutics that modulate PD-1- mediated inhibition of T-cell activation.
- The PathHunter Jurkat PD-1 Pathway Reporter Assay offers an additional option for testing PD-1 therapeutics to complement results from our PathHunter Jurkat PD-1 Signaling Assay.
- The PathHunter Jurkat PD-1 Signaling Assay captures a proximal signaling event (via SHP-recruitment), while the PathHunter Jurkat PD-1 Pathway Reporter Assay quantifies downstream effects of PD-1 (that require T-cell activation). Both assays produce robust and sensitive responses when tested with PD-1 antagonist antibodies.

Learn more about the Eurofins DiscoverX PathHunter Signaling Pathway Reporter Assays at discoverX.com/Reporters

© 2021 Eurofins DiscoverX, a Eurofins Discovery Company. All Rights Reserved. 21247 061621