

PathHunter® Jurkat PD-1 Signaling Reporter Cell Line

Catalog Number: 93-1153C19

Lot Number: See Vial

Contents: 1 x 10⁶ cells per vial in 1 mL

Background

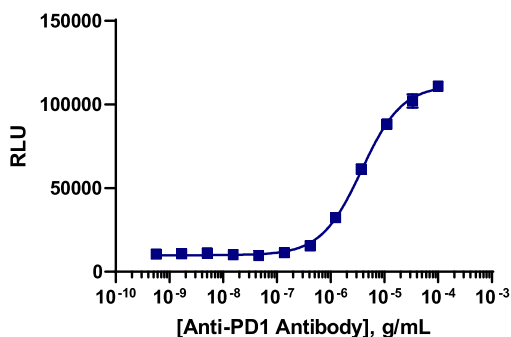
PathHunter Pathway Reporter cell lines are engineered to express an Enzyme Donor (ED) tagged reporter protein controlled by a pathway-inducible transcriptional response element. Pathway activation (through an endogenous or heterologously expressed receptor) results in induced expression of the ED-tagged protein. For inhibitory checkpoint receptor assays, co-expression of the relevant inhibitory receptor in Jurkat reporter cells attenuates T Cell Receptor (TCR) activation mediated by co-culture with the appropriate checkpoint ligand/TCR activator cell line. Treatment of these reporter cells with an inhibitor of the checkpoint receptor results in an increase in TCR activation. After drug treatment, changes in pathway reporter activity are detected by the addition of exogenous Enzyme Acceptor (EA) and lysis buffer and enzyme substrate, which forces complementation of the ED and EA enzyme fragments. This results in the formation of a functional enzyme that hydrolyzes its substrate to generate a chemiluminescent signal.?

Product Information

Target Receptor: PD-1
Target Accession #: NM_005018.2
Description: Programmed cell death protein 1
Species: Human
Response Element NFAT
Reporter ED Tag ePL
Cell Type: Jurkat (Suspension)
Storage: Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen.

Functional Performance

Pathway Reporter assay cells were seeded in a 96-well plate and then pre-incubated with a control antibody, followed by co-incubation with the ligand cell line using the assay conditions described below. Following stimulation, signal was detected using the PathHunter Detection Kit according to the recommended protocol. Please refer to page 2 for recommended assay reagents, detection reagents, and control compounds.



Signaling Assay Cell Number/Well: 10,000
Control Antibody: Anti-PD1 Antibody [NAT 105]
Antibody Pre-Incubation Time (minutes): 60
Antibody Pre-Incubation Temperature (°C): 37
IC50 for Antibody (ng/mL): 3750
Signal:Background: 10.7
Control Ligand Cell Line: PathHunter U2OS PD-L1/TCR Activator Ligand
Ligand Cell Number/Well: 15,000
Receptor:Ligand Cell Ratio: 1:1.5
Ligand Incubation Time (minutes): 300
Ligand Incubation Temperature (°C): 37
Detection Incubation Time (hrs.) 16

Jurkat cultures at time of plating for assay should be between 0.6-0.9 million cells/mL. Incubate with detection reagent mix overnight (~16 hours) for optimal S:B ratio.

Passage Stability

This cell line has been confirmed to be stable through a minimum of 10 passages with no significant drop in assay window or change in EC₅₀.

Mycoplasma Testing

This lot was tested and found to be free of mycoplasma contamination. Data available upon request.

Required Materials

The following additional materials are required but not provided:

Product Use*	Product Description	Catalog Number
Detection	PathHunter [®] ProLabel [®] /ProLink [™] Detection Kit	93-0812
Cell Culture	AssayComplete [™] Cell Culture Kit-101	92-3101G
Cell Plating	AssayComplete [™] Cell Plating 0 Reagent	93-0563R0A
Cell Detachment	Not Applicable	Not Applicable
Cell Thawing	AssayComplete [™] Thawing Reagent T6	92-4106TR
Cell Freezing	AssayComplete [™] Freezing Reagent F5	92-5105FR

*Please inquire about our cell line-specific AssayComplete Starter Packs to get you started with your cell culture needs.

Required Antibiotics

Antibiotic Name	Concentration (µg/mL)	Catalog Number
AssayComplete [™] Puromycin	0.25	92-0028
AssayComplete [™] Hygromycin B	250	92-0029
AssayComplete [™] G418	Not Applicable	Not Applicable

Additional Ligand Information

To be used with: PathHunter U2OS PD-L1/TCR Activator Ligand Cell Line (93-1154C3)

Control Antibody: Anti-PD1 Antibody [NAT 105]

Antibody Vendor: DiscoverX® (Part# 92-1290)

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