

PathHunter® Jurkat mPD-1 Signaling Assay

Catalog Number: 93-1158C19

Lot Number: See Vial

Contents: 3 x 10⁶ cells per vial in 1 mL

Background

Checkpoint Signaling assays are engineered to co-express a ProLink™ (PK) tagged immune checkpoint receptor and an Enzyme Acceptor (EA) tagged SH2 domain. Ligand engagement leads to receptor activation and phosphorylation, resulting in SH2-EA recruitment to the receptor, and forcing complementation of the two β-galactosidase enzyme fragments (EA and PK). The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

Product Information

Target Receptor: mPD-1

Target Accession #: NM_008798

Description: Programmed cell death protein 1

Species: Mouse

SH2 Domain: SHP1

SH2 Accession #: NM_080548

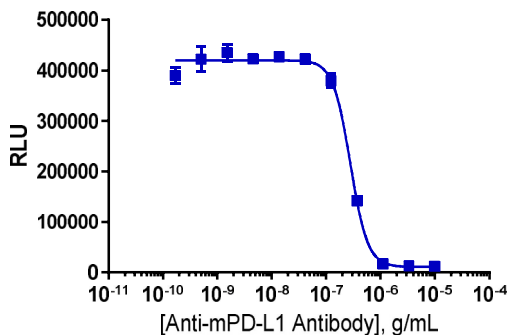
Amino Acid Range: 1 - 219

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

Signaling 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:	20,000
Control Antibody:	Anti-mPD-L1 Antibody
Antibody Pre-Incubation Time (minutes):	60
Antibody Pre-Incubation Temperature (°C):	37
IC50 for Antibody (ng/mL):	280
Signal:Background:	31.9
Control Ligand Cell Line:	PathHunter® U2OS mPD-L1 Ligand Cell Line
Ligand Cell Number/Well:	30,000
Receptor:Ligand Cell Ratio:	2:3
Ligand Incubation Time (minutes):	120
Ligand Incubation Temperature (°C):	RT
Detection Incubation Time (hrs.)	1

Note: For this assay, U2OS mPD-L1 Ligand cells were seeded first and pre-incubated for 1 hour at 37°C with Anti-mPD-L1 Antibody, followed by co-culture with the Jurkat mPD-1 Signaling cells.

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® Bioassay Detection Kit	93-0933
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	Not Applicable	Not Applicable
AssayComplete™ Hygromycin B	250	92-0029
AssayComplete™ G418	500	92-0030

Additional Ligand Information

To be used with: PathHunter® U2OS mPD-L1 Ligand Cell Line (93-1159C3)

Control Antibody: Anti-mPD-L1 Antibody

Antibody Vendor: Eurofins DiscoverX® (Part#: 92-1353)

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