

PathHunter® Jurkat CTLA4 Signaling Assay

Catalog Number: 93-1094C19

Lot Number: See Vial

Contents: 2 x 10⁶ cells per vial in 1 mL

Background

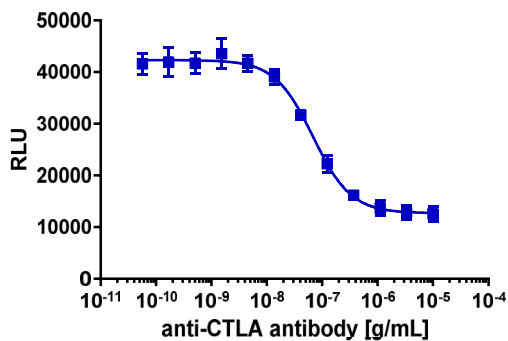
Checkpoint Signaling assays are engineered to co-express a ProLink™ (PK) tagged immune checkpoint receptor and an Enzyme Acceptor (EA) tagged SH2 domain. Ligand engagement leads to receptor activation and phosphorylation, resulting in SH2-EA recruitment to the receptor, and forcing complementation of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

Product Information

Target Receptor: CTLA4 (Chimeric)
Target Accession #: NM_005214.4
Description: Cytotoxic T-lymphocyte protein 4
Species: Human
SH2 Domain: SHP1
SH2 Accession #: NM_002831
Amino Acid Range: 1 - 161
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-CTLA4 Antibody [14D3]
Antibody Pre-Incubation Time (minutes): 60
Antibody Pre-Incubation Temperature (°C): 37
IC50 for Antibody (ng/mL): 68.2
Signal:Background: 3.5
Control Ligand Cell Line: PathHunter® U2OS CD86 Ligand Cell Line
Ligand Cell Number/Well: 30,000
Receptor:Ligand Cell Ratio: 2:3
Ligand Incubation Time (minutes): 240
Ligand Incubation Temperature (°C): 37
Detection Incubation Time (hrs.) 1

Additional Receptor Information

This assay employs a chimeric receptor composed of the extracellular CTLA4 domain (aa 1-161) fused to the PD-1 transmembrane and cytosolic domains (aa 171-288)

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 CD86 Ligand Cell Line (93-1093C3)

Control Antibody: Anti-CTLA4 Antibody [14D3]

Antibody Vendor: Eurofins DiscoverX® (Part#: 92-1319)

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