

# PathHunter® Jurkat CD28 Signaling Assay

## Materials Provided

**Catalog Number:** 93-1126C19

**Lot Number:** See Vial

## Background

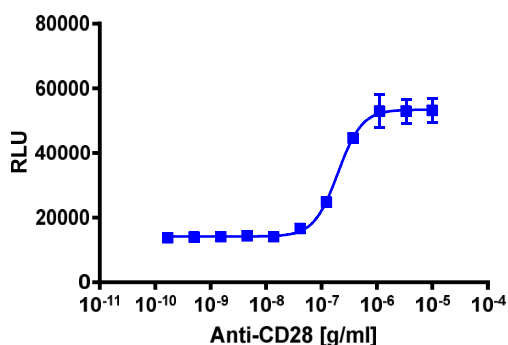
Checkpoint Signaling assays are engineered to co-express a ProLink™ (PK) tagged immune checkpoint receptor and an Enzyme Acceptor (EA) tagged SH2 domain. Ligand engagement leads to receptor activation and phosphorylation, resulting in SH2-EA recruitment to the receptor, and forcing complementation of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

## Product Information

**Target Receptor:** CD28  
**Target Accession #:** NM\_006139.3  
**Description:** T-cell-specific surface glycoprotein CD28  
**Species:** Human  
**SH2 Domain:** GRB2  
**SH2 Accession #:** NM\_002086.4  
**Amino Acid Range:** 60 - 152  
**Cell Type:** Jurkat (Suspension)  
**Contents:** 3 x 10<sup>6</sup> cells per vial in 1 mL  
**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 incubated at 37°C and 5% CO<sub>2</sub> for the indicated time specified below. Following agonist incubation, 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 Cell Number/Well:** 50000  
**Signaling Cell Seeding Time (hours):** 0  
**Control Agonist:** Anti-CD28 Antibody[CD28.2]  
**Agonist Incubation Time (minutes):** 120  
**Agonist Incubation Temperature (°C):** RT  
**EC<sub>50</sub> for compound stimulation (ng/mL):** 198.1  
**Signal:Background at agonist E<sub>max</sub>:** 3.8

**Detection Incubation Time (hrs.)** 1

### 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 EC<sub>50</sub>.

### 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

**Control Agonist:** Anti-CD28 Antibody[CD28.2]

**Vendor:** Eurofins DiscoverX® (Catalog No. 92-1336)

### Limited Use License Agreement

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