

PathHunter® U2OS Siglec9 Internalization Cell Line

Catalog Number: 93-1147C3

Lot Number: See Vial

Contents: 1 x 10⁶ cells per vial in 1 mL

Background

PathHunter Receptor Internalization cell lines are engineered to co-express the ProLink™ (PK) tagged receptor, and an Enzyme Acceptor (EA) tag localized to the endosomes. Pre-complexing a receptor-specific antibody with a secondary antibody stimulates robust internalization of the receptor-PK fusion protein upon antibody binding, resulting in co-localization of the receptor/antibody complex with EA-tagged endosomes, and forcing complementation of the two β-galactosidase (β-gal) enzyme fragments (EA and PK). The resulting functional β-gal enzyme hydrolyzes substrate to generate a chemiluminescent signal.

Product Information

Target Receptor: Siglec9

Description: Sialic acid-binding Ig-like lectin 9

Accession Number: NM_014441.2

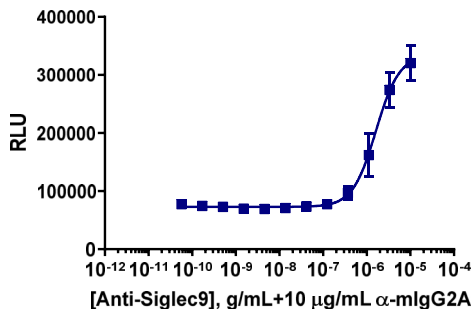
Target Species: Human

Cell Type: U2OS

Storage: Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen.

Functional Performance

Cells were plated in a 96-well plate and incubated at 37°C and 5% CO₂ for the indicated time specified below. Prior to addition to the cells, agonist antibody was pre-incubated with an appropriate secondary antibody at room temperature for 30-60 minutes. Cells were then stimulated with a dilution series of the cross-linked antibody, using the assay conditions specified 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.



Cell Number/Well: 5000

Cell Seeding Time (hours): 24

Control Antibody: Anti-Siglec9 Antibody

Antibody Isotype (Species): IgG2a (mouse)

Assay Incubation Time (minutes): 300

Assay Incubation Temperature (°C): 37

EC₅₀ for Antibody Stimulation (ng/mL): 1624

Signal:Background at Agonist E_{max}: 4.6

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 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® Flash Detection Kit	93-0247
Cell Culture	AssayComplete™ Cell Culture Kit-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 5 Reagent	93-0563R5A
Cell Detachment	AssayComplete™ Cell Detachment Reagent	92-0009
Cell Thawing	AssayComplete™ Thawing Reagent T3	92-4103TR
Cell Freezing	AssayComplete™ Freezing Reagent F3	92-5103FR

*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-Siglec9 Antibody

Vendor: Eurofins DiscoverX® (Catalog No. 92-1357)

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