

## PathHunter® FcgRIIIa Clustering Cell Line (U20S)

**Catalog Number:** 93-1184C3

**Lot Number:** see vial.

**Contents:** 1 x 10<sup>6</sup> cells per vial in 1 mL

### Background

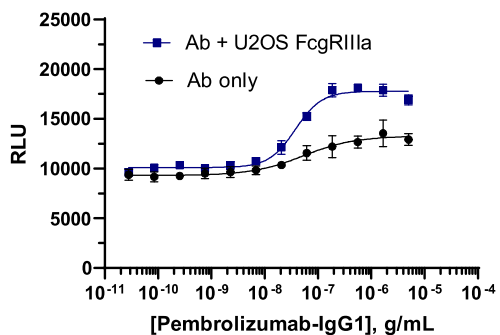
Checkpoint Clustering Cell Lines are companion products to the Checkpoint Signaling Assays that have been engineered to exogenously express a specific Fcg receptor. When co-cultured with an appropriate Checkpoint Signaling Cell Line in the presence of antigen-specific agonist antibodies, the Fcg receptor binds to the Fc portion of the antibody, presenting the antigen binding end to the checkpoint receptor on the Checkpoint Signaling Cell Line. This results in receptor clustering and robust receptor activation, leading to downstream signaling events, which are detected by β-galactosidase complementation. The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

### Product Information

**Target:** FcgRIIIa  
**Accession #:** AAH36723.1  
**Description:** Low affinity immunoglobulin gamma Fc region receptor III-A  
**Species:** Human  
**Cell Type:** U2OS (Adherent)  
**Storage:** Short term (<24 h): Store at -80°C; Long term (>24 h): Store in vapor phase of liquid nitrogen.

### Functional Performance

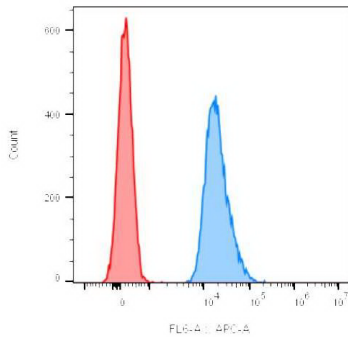
Fcg receptor cells were seeded in a 96-well plate and then pre-incubated with a control antibody, followed by co-incubation with the signaling assay cell line using the assay conditions described below. Following stimulation, signal was detected using the PathHunter Detection Kit according to the recommended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



**Clustering Cell Number/Well:** 20000  
**Control Antibody:** Anti-hPD1-Pem-hIgG1  
**Antibody Pre-Incubation Time (minutes):** 60  
**Antibody Pre-Incubation Temperature (°C):** 37  
**Signaling Assay:** PathHunter® Jurkat PD-1 (SHP1) Signaling Cell Line  
**Signaling Assay Cell Number/Well:** 5000  
**Target:Clustering Cell Ratio:** 2:1  
**Signaling Cell Inc Time (minutes):** 120  
**Signaling Cell Temperature (°C):** 22  
**EC<sub>50</sub> for Antibody (ng/mL):** 37.7  
**Signal:Background at Agonist E<sub>max</sub>:** 1.9  
**Detection Incubation Time (hrs.)** 1

Clustering cells should be passaged 2X after thaw prior to use. Assay plate can also be re-read after overnight detection.

### Cell Line Fcg Receptor Expression



Fcg receptor clustering cells were immunostained for receptor expression and analyzed by flow cytometry. Red, native U2OS; blue, U2OS Fcg receptor clustering cell line.?

### Passage Stability

This cell line has been confirmed to be stable through a minimum of 10 passages with no significant drop in expression levels.

### 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
Cell Culture	AssayComplete™ Cell Culture Kit-103	92-3103G
Cell Plating	AssayComplete™ Cell Plating 0 Reagent	93-0563R0A
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	0.25	92-0028
AssayComplete™ Hygromycin B	Not Applicable	Not Applicable
AssayComplete™ G418	Not Applicable	Not Applicable

**To be used with:** PathHunter® Jurkat PD-1 (SHP1) Signaling Cell Line (93-1104C19)

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