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## **Data Sheet**

### **CD155 (PVR) - HEK293 Cell Line**

### **Catalog #60537**

**Background:** CD155 is a transmembrane glycoprotein receptor belonging to the immunoglobulin superfamily. It is highly expressed on dendritic cells, fibroblasts, endothelial cells, and multiple tumor cells including ovarian carcinoma, non-small cell lung, glioblastoma, and colorectal carcinoma. It has a high affinity for a T cell regulatory transmembrane surface protein called TIGIT (T cell immunoglobulin and ITIM domains), and plays a key role in tumor cell invasion and migration. Interaction between CD155 and TIGIT causes immunosuppressive effects on CD4+ and CD8+ T cells, which hinders T cell proliferation and function. CD155 also interacts with regulatory receptors CD226 (expressed on natural killer (NK) cells, monocytes and CD4+ T cells) and CD96. While CD155-CD226 engagement activates NK cell cytotoxicity and T cell response, CD155-CD96 interaction inhibits NK cell function. Due to these opposing regulatory effects, blockade of CD155-TIGIT and CD155-CD226 interactions are attractive therapeutic strategies in cancer immunotherapy and autoimmune diseases, respectively.

**Description:** Recombinant HEK293 cell line expressing full length human CD155, also known as poliovirus receptor (PVR) or nectin-like molecule-5 (NECL-5). Expression is confirmed by Western Blot.

**Application:** Since the HEK293 parental cell line does not express endogenous CD155, the CD155/HEK293 Cell Line functions as an antigen-presenting cell (APC) to stimulate TIGIT-specific signaling responses in lymphocytes, such as cell proliferation and cytokine release, or with a TIGIT reporter cell line (BPS Cat. #60538). This cell line is also ideal for validating binding affinity to TIGIT (see application reference 1) or CD155 and CD226 (see application reference 2).

#### **Host Cell**

HEK293 cells; adherent epithelial cells.

#### **Format**

Each vial contains ~3 x 10<sup>6</sup> cells in 1 ml of 10% DMSO in FBS.

#### **Storage**

Store in liquid nitrogen immediately upon receipt.

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### Culture Medium

**Thaw Medium 1 (BPS Cat. #60187):** MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

**Growth Medium 1F (BPS Cat. # 79540):** Thaw Medium 1 (BPS Cat #60187) plus 100 µg/ml Hygromycin B (Thermo Fisher, Cat. #10687010).

### Culture conditions

**Frozen Cells:** Prepare a T-25 culture flask with 10 ml of pre-warmed growth medium. Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to Thaw Medium 1 (**no Hygromycin B**). Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO<sub>2</sub>. After 48 hours of incubation, change to fresh Thaw Medium 1 (**no Hygromycin B**), without disturbing the attached cells. Continue to change the medium every 2-3 days until the cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 1F after the first passage.

**Subculture:** When cells reach 90% confluency, remove the medium and GENTLY wash once with PBS (without Magnesium or Calcium). These cells are loosely adherent and detach easily so do not re-suspend the PBS directly onto the cell surface. Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed Growth Medium 1F and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and re-suspend cells in 10 ml of pre-warmed Growth Medium 1F. Dispense 5 ml of the cell suspension into a new T75 flask containing pre-warmed 20 ml Growth Medium 1F. Incubate cells in a humidified 37°C incubator with 5% CO<sub>2</sub>. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

### Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, Cat. #LT07-518) was used as a positive control.

### Application References

1. Yu, X., *et.al.* (2009) The Surface Protein TIGIT Suppresses T cell Activation by Promoting the Generation of Mature Immunoregulatory Dendritic Cells. *Nat Immunol.* **10**: 48-57.
2. Bottino, C., *et.al.* (2003) Identification of PVR (CD155) and Nectin-2 (CD112) as Cell Surface Ligands for the Human DNAM-1 (CD226) Activating Molecule. *J Exp Med.* **198**: 557-567.

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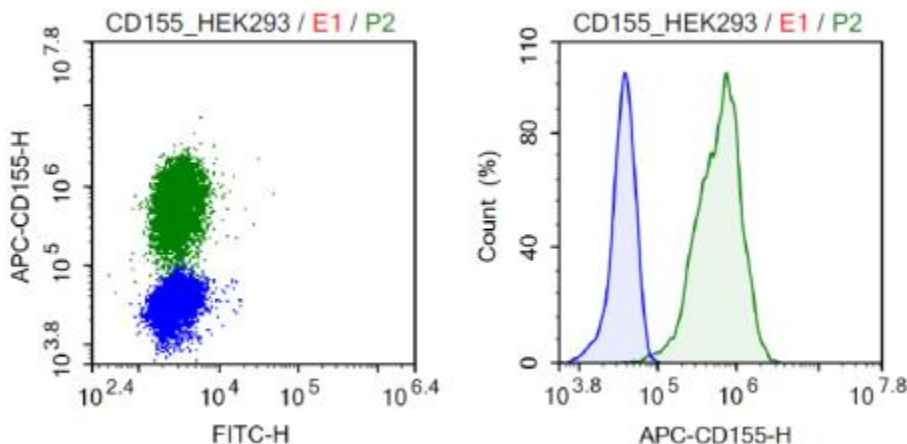
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3. Sloan, K.E., *et.al.* (2005) CD155/PVR Enhances Glioma Cell Dispersal by Regulating Adhesion Signaling and Focal Adhesion Dynamics. *Cancer Res.* **65**: 10930-37.

### Quality Assurance and Functional Analysis



**Figure 1. Expression of CD155 protein in CD155/HEK293 cells validated by flow cytometry.** Flow cytometry showed APC conjugated anti- human CD155 antibody (Biolegend, Cat. # 337617) detects CD155-positive cells (green), using naïve HEK293 cells as a negative control (blue).

### Vector and sequence

Human PVR/CD155 (NP\_001129241.1; Accession BC BC015542) was cloned into the MCS of pIRESHyg3 vector (Clontech, Cat No. 631620).

MARAMAAAWPLLLVALLVLSWPPPGTGDVVVQAPTQVPGFLGDSVTLP CYLQVPNMEVTHVS  
 QLTWARHGESGSMVAFHQ TQG PSYSESKRLEFVAARLG AELRNASLRMFGLRVEDEGNYTCL  
 FVTFPQGSRSVDIWLRLAKPQNTAEVQKVQLTGEPVPMARCVSTGGRPPAQITWHS DLGGM  
 PNTSQVPGFLSGTVT VTS LWILVPSSQVDGKNVTCKVEHESFEKPQLLTVNLTVYYPPEVSISG  
 YDNNWYLGQNEATLTCDARSNPEPTGYNWSTTMGPLPPFAVAQGAQLLIRPVDKPINTTLCN  
 VTNALGARQAELTVQVKEGPPSEHSGMSRNAIFLV LGILVFLILLGIGIYFYWSKCSREVLWHC  
 HLCPSSTEHASASANGHVSYS AVSRENSSSQDPQTEGTR

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## Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Thaw Medium 1	60187	100 ml
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Human CD155 (PVR) His-tag Protein	71181	100 µg
Mouse CD155 (PVR) His-tag Protein	71167	100 µg
Mouse CD155, His-tag, Biotin-labeled	71168	50 µg
Human CD226, Fc fusion	71252	100 µg
Human CD226, Fc fusion, Biotin-labeled	71253	50 µg
Human CD112, Fc fusion	71197	100 µg
Human CD112, Fc fusion, Biotin-labeled	71234	50 µg
Human TIGIT, Fc fusion	71186	100 µg
Human TIGIT, Fc fusion, Biotin-labeled	71251	50 µg
TIGIT:CD155 Homogenous Assay Kit	72029	384 reactions
CD226: CD155 Homogenous Assay Kit	72052	384 reactions
TIGIT:CD112 Homogenous Assay Kit	72030	384 reactions
CD226: CD112 Homogenous Assay Kit	72051	384 reactions
TIGIT NFAT-Luciferase Reporter Jurkat Cell Line	60538	2 vials

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