

Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) Data Sheet

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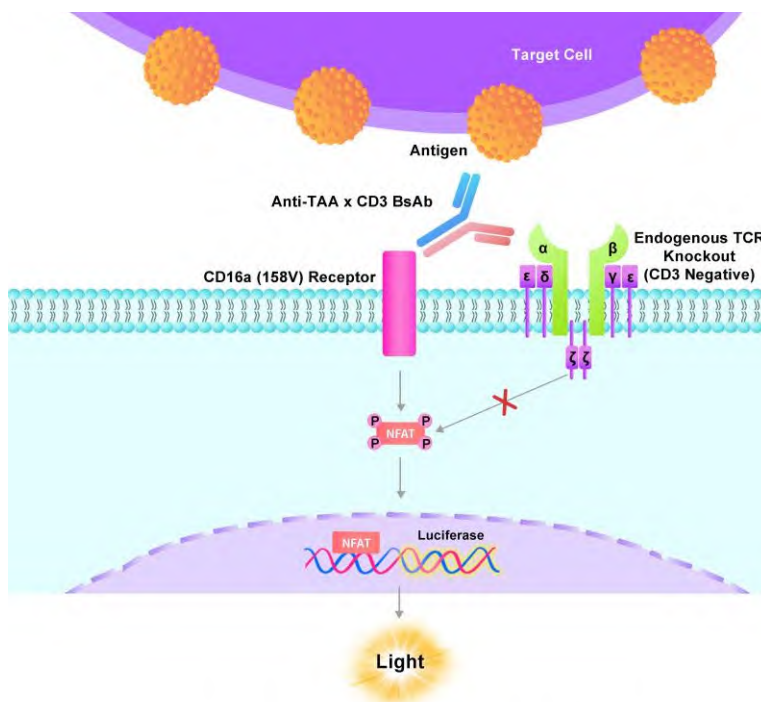
Catalog No.	Size
CJUR-STF299	2 × (1 vial contains ~5×10 ⁶ cells)

• Description

The Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) was engineered to not only express the NFAT signaling response element and the receptor full length human CD16a (Uniprot: P08637) mutated to a Valine (V) at amino acid 158 exhibiting a higher affinity for IgG1 and IgG3 isotypes compared to CD16a-158F, but also targeted knockout of the endogenous TCR chains resulting in loss of functional TCR/CD3 complex on the cell surface. When co-cultured with a target cell and the corresponding anti-TAA × CD3 bispecific antibody (BsAb), the anti-TAA × CD3 BsAb simultaneously binds both the target-associated antigen (TAA) on the target cell and the CD16a (158V) receptor on the Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative), resulting in receptor clustering, intracellular signaling and NFAT-mediated luminescence.

• Application

- Determination of ADCC activity induced by anti-TAA × CD3 BsAbs



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• Cell Line Profile

Cell line	Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative)
Host Cell	Jurkat
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS
Selection Marker	Puromycin (5 µg/mL) + Hygromycin (20 µg/mL) + Zeocin (20 µg/mL)
Incubation	37°C with 5% CO ₂
Doubling Time	16-20 hours

• Materials Required for Cell Culture

- RPMI Medium 1640 (Gibco, Cat. No. 11875-093)
- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)
- Zeocin ((Invitrogen, Cat. No. R25001)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Complete Growth Medium: RPMI-1640 + 10% FBS, 1%P/S
- Culture Medium: RPMI-1640 + 10% FBS, Puromycin (5 µg/mL), Hygromycin B (20 µg/mL), Zeocin (20 µg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO₂ Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium.
4. Count viable cells and centrifuge at approximately 1000 rpm for 5 minutes.
5. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh **complete growth medium**. Adjust the cell density of the suspension to 1×10^6 viable cells/mL and transfer cells to an appropriate size vessel.
6. Incubate at 37°C with 5% CO₂ incubator.

• *Subculture*

Cell viability may be low after thawing, and full recovery (viability >90%) may take up to 1-2 weeks. Once the cell density reaches approximately 2×10^6 viable cells/mL, adjust the density to a range of 2×10^5 - 5×10^5 viable cells/mL by either adding the fresh **culture medium** or replacing the existing culture medium. Avoid allowing the cell density to exceed 3×10^6 cells/mL, as this may negatively impact cell performance in subsequent passages. T-75 flasks are recommended for subculturing.

• **Subculturing Frequency:** It is recommended to subculture every 3-4 days, adjusting the frequency based on the cell density in your specific culture system.

Note: After recovery, maintain the cells for 1-2 passages in the **complete growth medium not containing the selection marker**, if the cells are in good condition (viability >90%), transition to the **culture medium containing the selection marker during subculturing**.

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• *Cryopreservation*

1. Count viable cells and harvest the cell suspension.
2. Centrifuge at 1000 rpm for 5 min at room temperature and resuspend cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
3. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a -80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a -80°C freezer immediately upon receipt. If stored in a -80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

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• *Receptor Assay*

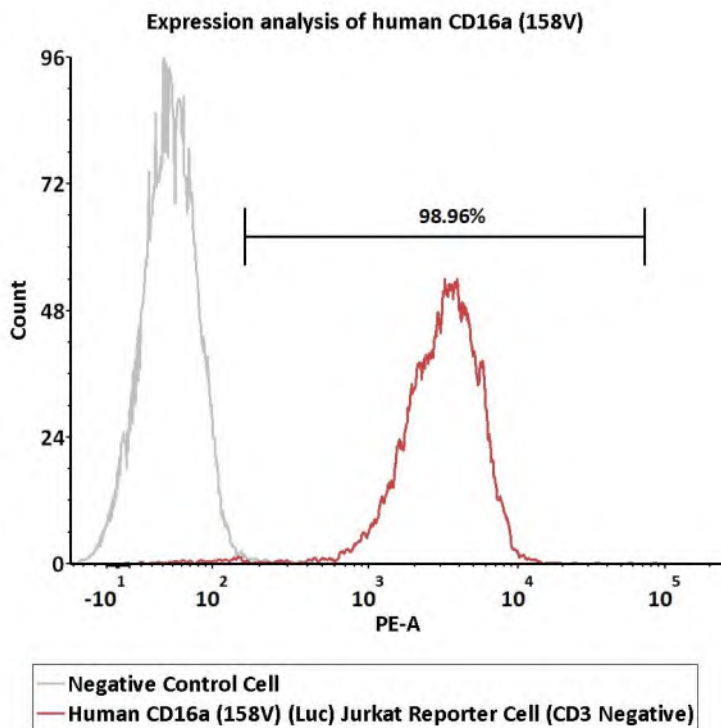


Fig1. Expression analysis of human CD16a (158V) on Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) by FACS. Cell surface staining was performed on Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) or negative control cell using PE-labeled anti-human CD16a antibody.

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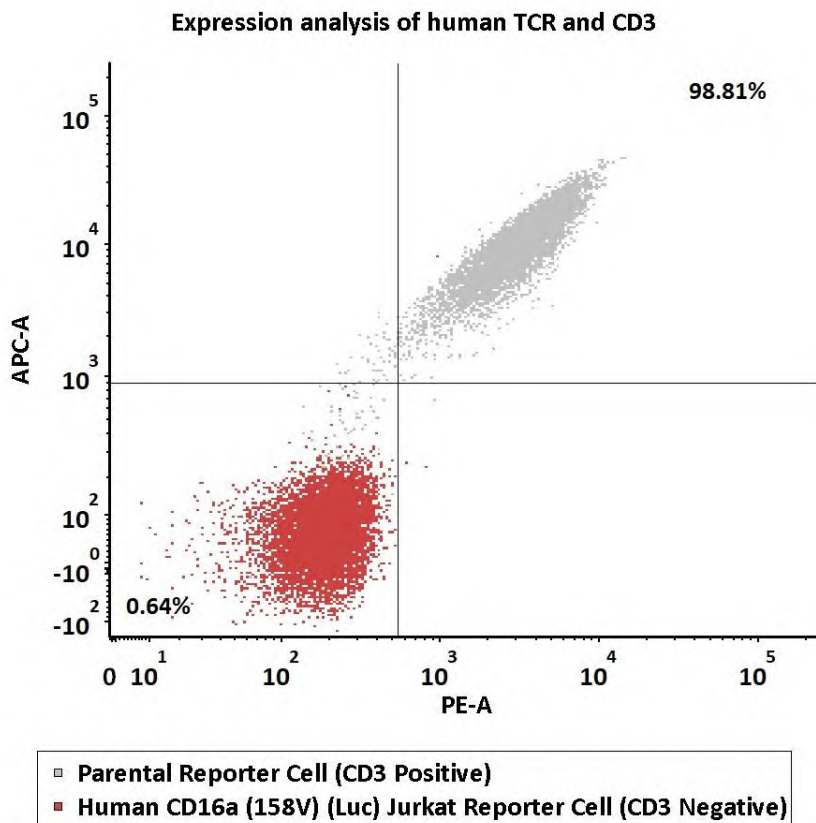


Fig2. Expression analysis of human TCR and human CD3 on Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) by FACS. Cell surface staining was performed on Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) using PE-labeled anti-human TCR antibody and APC-labeled anti-human CD3 antibody. The parental reporter cell (CD3 Positive) was used as the control. This result confirmed the loss of TCR/CD3 complex on the cell surface of Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative).

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• Signaling Bioassay

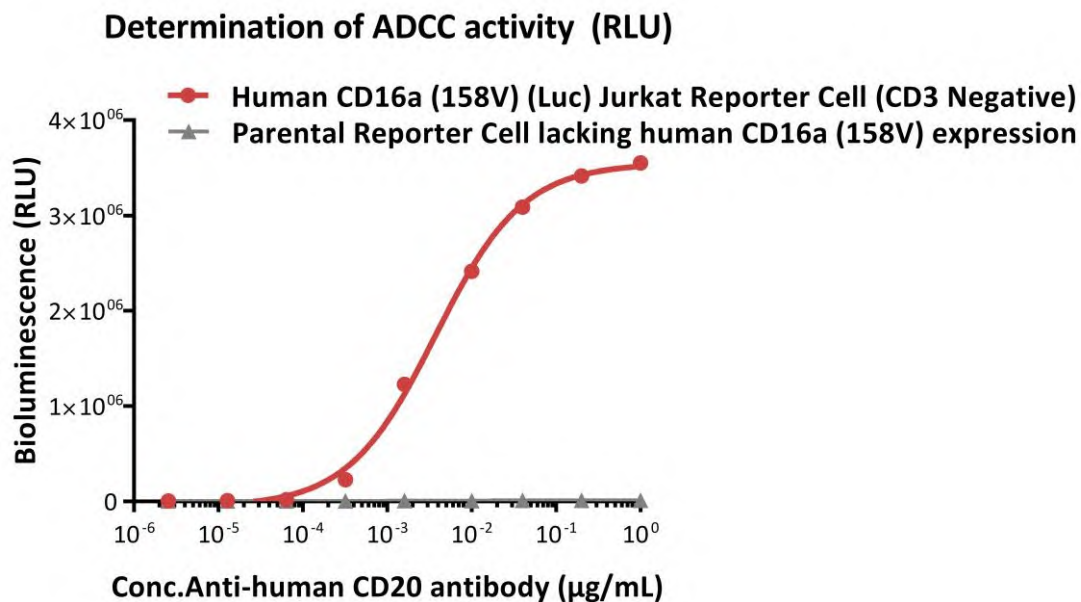


Fig3. ADCC response to anti-human CD20 antibody (RLU). Anti-human CD20 antibody-induced ADCC activity was evaluated using Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) in the presence of Raji cells that express CD20 endogenously. The parental reporter cell lacking human CD16a (158V) expression was used as the control. The EC50 of anti-human CD20 antibody on the Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) was approximately 0.0038 µg/mL.

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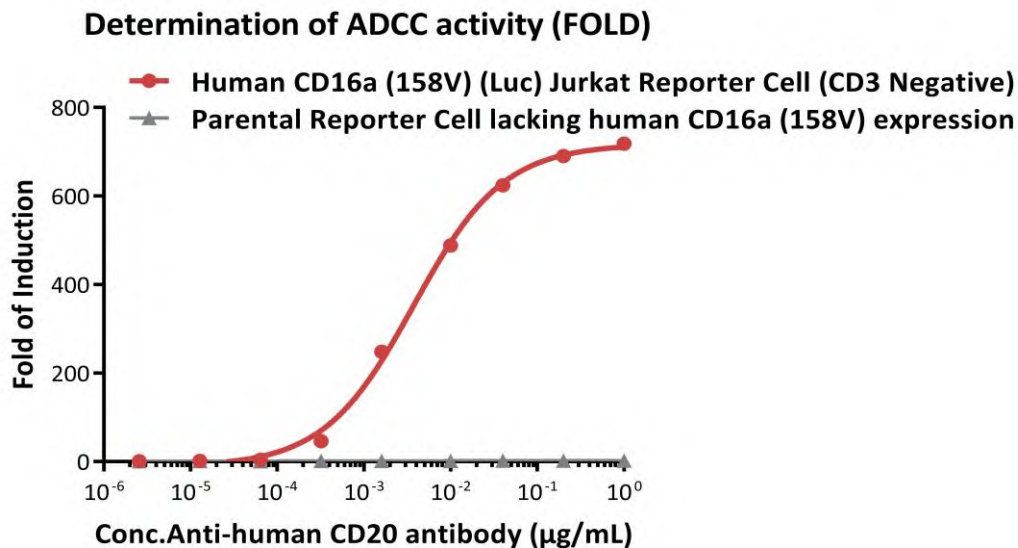


Fig4. ADCC response to anti-human CD20 antibody (FOLD). Anti-human CD20 antibody-induced ADCC activity was evaluated using Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) in the presence of Raji cells that express CD20 endogenously. The parental reporter cell lacking human CD16a (158V) expression was used as the control. The max induction fold on Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) was approximately 718.04.

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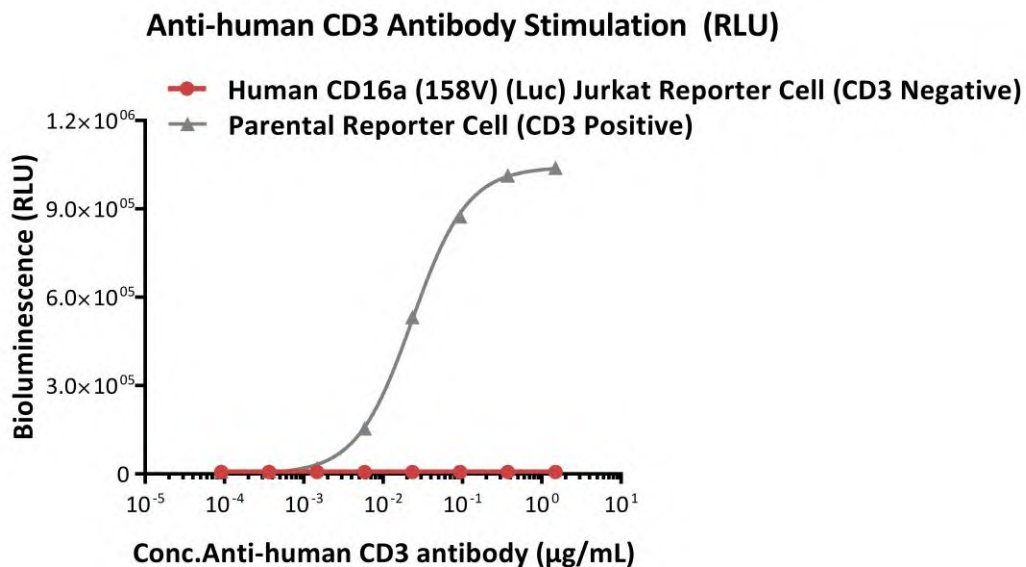


Fig5. Response to anti-human CD3 antibody (RLU). Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) was incubated with serial dilutions of anti-human CD3 antibody (Cat. No. CDE-M120a). The parental reporter cell (CD3 Positive) was used as the control. Compared to the parental reporter cell (CD3 Positive), the Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) exhibited no activation response to anti-human CD3 antibody stimulation.

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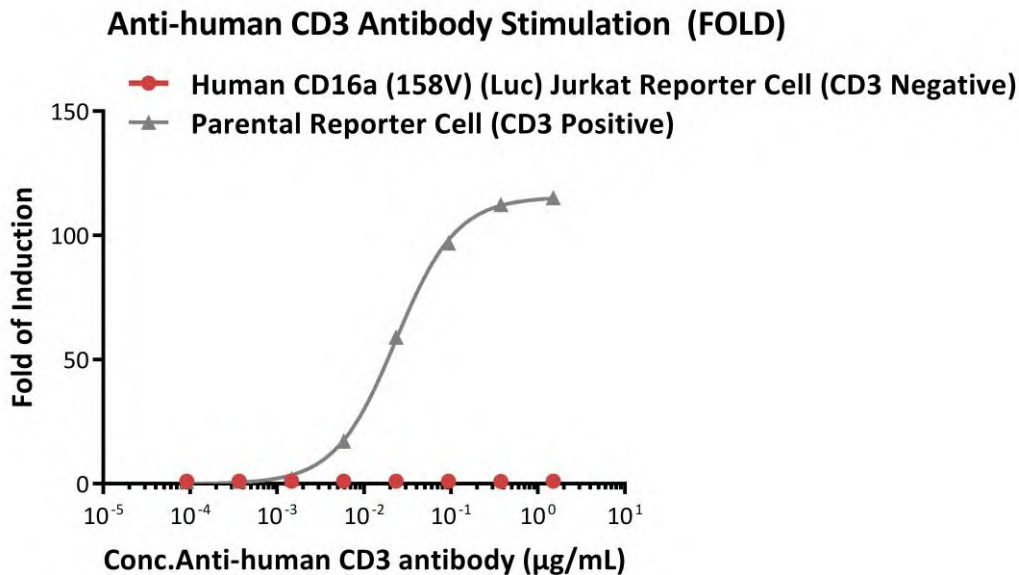


Fig6. Response to anti-human CD3 antibody (FOLD). Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) was incubated with serial dilutions of anti-human CD3 antibody (Cat. No. CDE-M120a). The parental reporter cell (CD3 positive) was used as the control. Compared to the parental reporter cell (CD3 Positive), the Human CD16a (158V) (Luc) Jurkat Reporter Cell (CD3 Negative) exhibited no activation response to anti-human CD3 antibody stimulation.

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• *Related Products*

<u>Products</u>	<u>Cat.No.</u>
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+)	CJUR-STF247
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+ CD8+)	CJUR-STK270
Raji/Human CD19 Knockout Stable Cell Line	SCRAJ-STT216
Raji/Human CD20 Knockout Stable Cell Line	SCRAJ-STT227
Raji/Human CD19 & CD20 Double Knockout Stable Cell Line	CRAJ-STK238
NFAT (Luc) Jurkat Reporter Cell	SCJUR-STF046
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
HEK293/Human CCR5 Stable Cell Line	CHEK-ATP043
HEK293/Human SIRP alpha Stable Cell Line	CHEK-ATP051
HEK293/Human CD20 Stable Cell Line	CHEK-ATP034
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human TMPRSS2-HA-P2A-mGFP Stable Cell Line	CHEK-ATP101
NF-kB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
NY-ESO-1 specific TCR-HEK293 cell line	CHEK-STP114
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
HEK293/Human CCR8 Stable Cell Line	CHEK-ATP140
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
Human BMP (Luc) HEK293 Reporter Cell	CHEK-ATF188
HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP199

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<u>Products</u>	<u>Cat.No.</u>
HEK293/Human IDH1(132R)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP200
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
Human GCGR (Luc) HEK293 Reporter Cell	CHEK-ATF103
Human GIPR (Luc) HEK293 Reporter Cell	CHEK-ATF104
Human HVEM (Luc) HEK293 Reporter Cell	CHEK-ATF105
Human HVEM (Luc) HEK293 Reporter Cell	CHEK-ATF105
Human BTLA (Luc) Jurkat Reporter Cell	SCJUR-STF106
Human IGF-1 R (Luc) HEK293 Reporter Cell	CHEK-ATF107
NF-kB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
Human GLP-2R (Luc) HEK293 Reporter Cell	CHEK-ATF128
Human 5-HT1A (Luc) HEK293 Reporter Cell	CHEK-ATF131
Human RANK (Luc) HEK293 Reporter Cell	CHEK-ATF129
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell	CHEK-ATF133
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
Human OX40 (Luc) HEK293 Reporter Cell	CHEK-ATF135
Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell	CHEK-ATF136
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
Human FGF-21 (Luc) HEK293 Reporter Cell	CHEK-ATF163
Human Activin RII (Luc) HEK293 Reporter Cell	CHEK-ATF164
Human IL-23 R/IL-12 R beta 1(Luc) HEK293 Reporter Cell	CHEK-ATF166
Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell	CHEK-ATF167
Human VEGF R2 (Luc) HEK293 Reporter Cell	CHEK-ATF044

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• *Related Products*

Products

Cat.No.

STAT3 (Luc) HEK293 Reporter Cell

CHEK-ATF047

NF-κB (Luc) HEK293 Reporter Cell

CHEK-ATF048

Human EGF R (Luc) HEK293 Reporter Cell

CHEK-ATF049