Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

➢ *Limited Use & License Disclosure*

BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE FOLLOWING TERMS OF LIMITED USE OF THIS CELL LINE PRODUCT.

- If the researcher is not willing to accept the terms of limited use of this cell line product, and the product is unused, ACRO will accept return of the unused product.
- Researchers may use this product for research use only, no commercial use is allowed. "Commercial use" means any and all uses of this product and derivatives by a party for profit or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research.
- This cell line is neither intended for any animal or human therapeutic purposes nor for any direct human in vivo use. You have no right to share, modify, transfer, distribute, sell, sublicense, or otherwise make the cell line available for use to other researchers, laboratories, research institutions, hospitals, universities, or service organizations.
- ACROBIOSYSTEMS MAKES NO WARRANTIES OR REPRESENTATIONS OF ANY KIND, EITHER EXPRESSED OR IMPLIED, WITH RESPECT TO THE SUITABILITY OF THE CELL LINE FOR ANY PARTICULAR USE.
- ACROBIOSYSTEMS ACCEPTS NO LIABILITY IN CONNECTION WITH THE HANDLING OR USE OF THE CELL LINE.
- Modifications of the cell line, transfer to a third party, or commercial use of the cell line may require a separate license and additional fees. Please contact order.cn@acrobiosystems.com for further details.

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

Catalog No.	Size
CJUR-STP292	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Jurkat/human TRBV9 & Luc Stable Cell Line was engineered to express the exogenous TCRβ chain harboring the human TRBV9 variable domain (NCBI: NG_001333.2) following knockout of the endogenous TCRβ chain. This reconstituted TRBV9-containing TCRβ chain pairs with the endogenous TCRα chain to reform the TCRαβ heterodimer, which subsequently assemble with the endogenous CD3 to enable cell surface expression of the TCR/CD3 complex. Surface expression of human TRBV9 was confirmed by flow cytometry. The endogenous TCRβ knockout was confirmed by genomic sequencing. This cell was also engineered to express the firefly luciferase reporter. The luciferase activity was confirmed by the detection of luminescence signal.

• *Application*

- Useful for cell-based TRBV9 binding assay
- Used as target cells for luminescence-based cytotoxicity evaluation

• *Cell Line Profile*

Cell line	Jurkat/Human TRBV9 & Luc Stable Cell Line
Host Cell	Jurkat
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin B (20 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	16-20 hours

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

• *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)

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 (2 μg/mL), Hygromycin B (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)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

• *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% CO_2 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.

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

• *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.

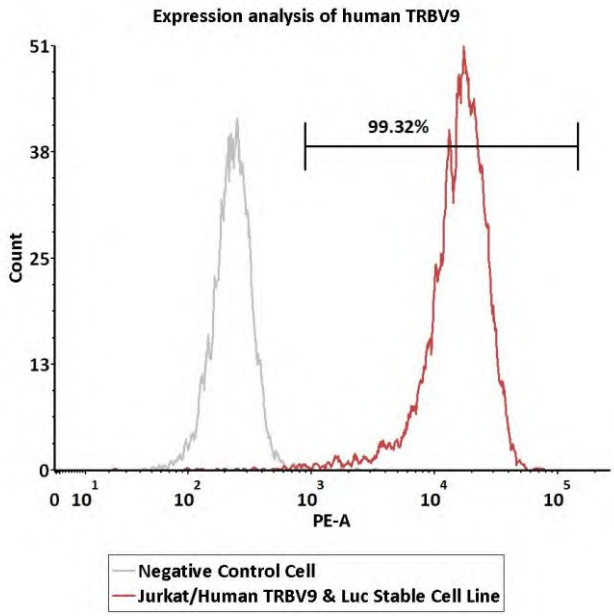
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *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.

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

- ***Receptor Assay***

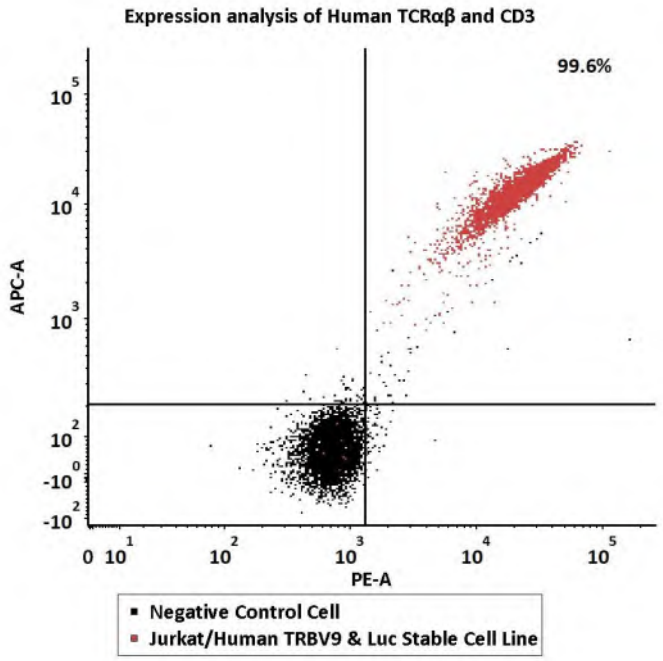


Catalog No.	Stable Cell Line	MFI for TRBV9 (PE)
NA	Negative Control Cell	227.05
CJUR-STP292	Jurkat/Human TRBV9 & Luc Stable Cell Line	16098.25

Fig1. Expression analysis of human TRBV9 on Jurkat/Human TRBV9 & Luc Stable Cell Line by FACS. Cell surface staining was performed on Jurkat/Human TRBV9 & Luc Stable Cell Line or negative control cell using anti-human TRBV9 antibody.

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for TCR αβ (PE)	MFI for CD3 (APC)
NA	Negative Control Cell	825.79	77.01
CJUR-STP292	Jurkat/Human TRBV9 & Luc Stable Cell Line	21847.09	13409.04

Fig2. Expression analysis of human TCRαβ and human CD3 on Jurkat/Human TRBV9 & Luc Stable Cell Line by FACS. Cell surface staining was performed on Jurkat/Human TRBV9 & Luc Stable Cell Line using PE-labeled anti-human TCR αβ antibody and APC-labeled anti-human CD3 antibody. The Jurkat/Human TRBV9 & Luc Stable Cell Line was stained with PE-labeled isotype control antibody and APC-labeled isotype control antibody as the negative control cell. This result demonstrated the reconstituted TRBV9-containing TCRβ chain paired with the endogenous TCRα can assemble with the endogenous CD3 to enable cell surface expression of the TCR/CD3 complex.

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

- ***Luminescence Assay***

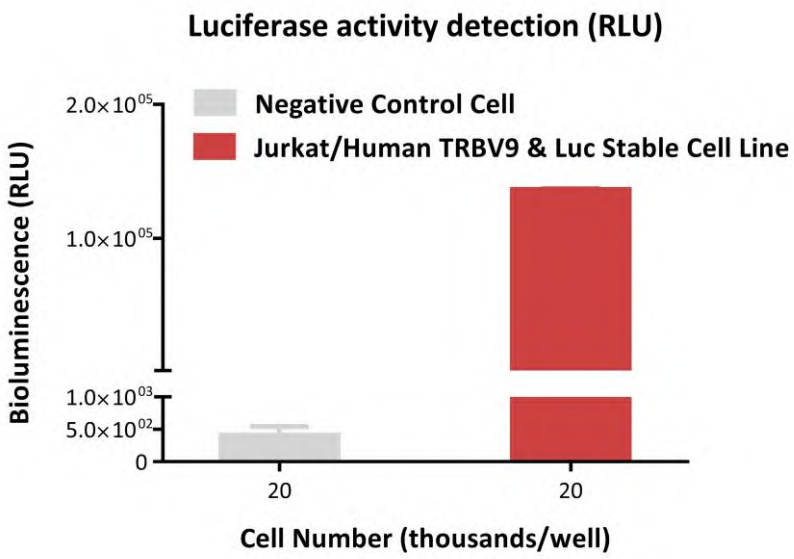


Fig3. Expression analysis of luciferase on Jurkat/Human TRBV9 & Luc Stable Cell Line (RLU). The luminescence signal of Jurkat/Human TRBV9 & Luc Stable Cell Line were detected by incubating with the luciferase substrate. The RLU value was approximately $1.36x10^5$ at the density of $2x10^4$ cells/well.

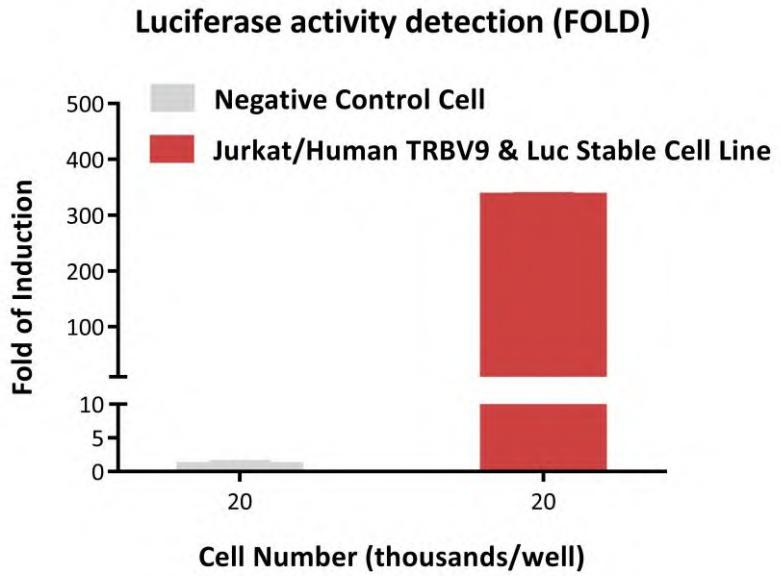


Fig4. Expression analysis of luciferase on Jurkat/Human TRBV9 & Luc Stable Cell Line (FOLD). The luminescence signal of Jurkat/Human TRBV9 & Luc Stable Cell Line were detected by incubating with the luciferase substrate. The max induction fold was approximately 335.55 at the density of $2x10^4$ cells/well.

- ***Sequencing Analysis***

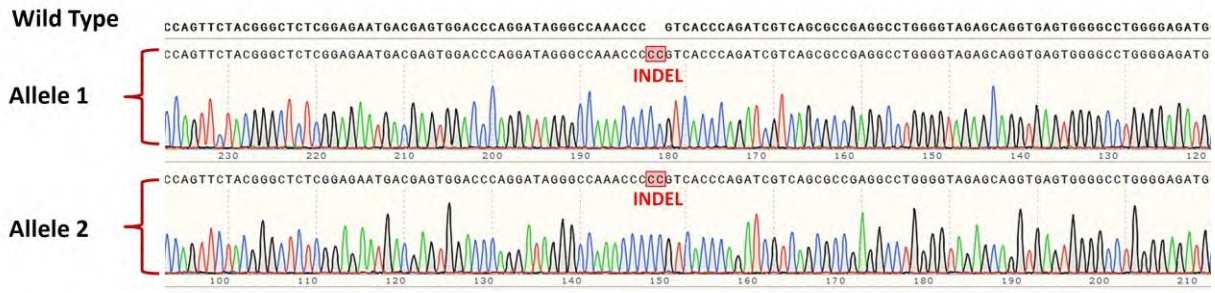


Fig5. Confirmation of endogenous human TCRβ knockout in the Jurkat/Human TRBV9 & Luc Stable Cell Line. Sanger sequencing was used for mutation analysis of human TRBC. The sequencing results demonstrated that frameshift mutations were generated in the human TRBC gene in the Jurkat/Human TRBV9 & Luc Stable Cell Line.

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

• *Related Products*

Products	Cat.No.
Jurkat/Human TRBV9 Stable Cell Line	CJUR-STP293
Raji/Luc & Human CD19 Knockout Stable Cell Line	CRAJ-STK286
Raji/Luc & Human CD20 Knockout Stable Cell Line	CRAJ-STK285
Raji/Luc & Human CD19 & CD20 Double Knockout Stable Cell Line	CRAJ-STK284
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
HEK293/Human TROP-2 Stable Cell Line	CHEK-ATP036
HEK293/Human Nectin-4 Stable Cell Line	CHEK-ATP035
HEK293/Human Anti-CD19 Stable Cell Line	CHEK-ATS056
CHO/Human GPRC5D Stable Cell Line	CCHO-STP078
HEK293/Human CEACAM5 Stable Cell Line	CHEK-ATP083
HEK293/Human ROR1 Stable Cell Line	CHEK-ATP084
HEK293/Human Transferrin R Stable Cell Line	CHEK-ATP089
HEK293/Human DLL3 Stable Cell Line	CHEK-ATP090
HEK293/Human FOLR1 Stable Cell Line	CHEK-ATP091
HEK293/Human Glypican-3 (GPC3) Stable Cell Line	CHEK-ATP092
CHO/Human DLL3 Stable Cell Line	SCCHO-ATP111
CHO/Human Glypican-3 (GPC3) Stable Cell Line	SCCHO-ATP112
HEK293/Human Transferrin Stable Cell Line	CHEK-ATP115
HEK293/Human NAPI-IIb Stable Cell Line	CHEK-ATP116
HEK293/Human Mesothelin Stable Cell Line	CHEK-ATP119
CHO/Human Mesothelin Stable Cell Line	SCCHO-ATP120
CHO/Human STEAP1 Stable Cell Line	SCCHO-ATP121
HEK293/Human ENPP3 Stable Cell Line	CHEK-ATP122
HEK293/Human LRRC15 Stable Cell Line	CHEK-ATP123

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

• *Related Products*

Products	Cat.No.
HEK293/Human Integrin alpha V beta 6 Stable Cell Line	CHEK-ATP125
HEK293/Human B7-H4 Stable Cell Line	CHEK-ATP126
HEK293/Human Cadherin-6 Stable Cell Line	CHEK-ATP127
CHO/Human c-MET Stable Cell Line	SCCHO-ATP141
HEK293/Human c-MET Stable Cell Line	CHEK-ATP146
HEK293/Human EGF R Stable Cell Line	CHEK-ATP148
HEK293/Human ErbB3 Stable Cell Line	CHEK-ATP149
HEK293/Human ErbB2 Stable Cell Line	CHEK-ATP150
HEK293/Human uPAR Stable Cell Line	CHEK-ATP151
CHO/Human uPAR Stable Cell Line	SCCHO-ATP152
HEK293/Human CD19 Stable Cell Line	CHEK-ATP003
HEK293/Human STEAP1 Stable Cell Line	CHEK-ATP154
CHO/Human B7-H3 (4Ig) Stable Cell Line	SCCHO-ATP169
CHO/Human CD79A&CD79B Stable Cell Line	SCCHO-ATP170
CHO/Human CD79B Stable Cell Line	SCCHO-ATP171
HEK293/Human Cadherin-17 Stable Cell Line	CHEK-ATP173
HEK293/Human EpCAM Stable Cell Line	CHEK-ATP175
HEK293/Human TPBG Stable Cell Line	CHEK-ATP176
CHO/Cynomolgus Glypican-3 (GPC3) Stable Cell Line	SCCHO-ATP179
HEK293/Human GUCY2C Stable Cell Line	CHEK-ATP182
HEK293/Human SEZ6 Stable Cell Line	CHEK-ATP183
HEK293/Human FAP Stable Cell Line	CHEK-ATP184
HEK293/Human PSMA Stable Cell Line	CHEK-ATP185
HEK293/Human PTK7 Stable Cell Line	CHEK-ATP186
HEK293/Human MCAM Stable Cell Line	CHEK-ATP195
NF-κB (Luc) HEK293 Reporter Cell	CHEK-ATF048

Jurkat/Human TRBV9 & Luc Stable Cell Line Data Sheet

• *Related Products*

Products	Cat.No.
HEK293/Human GPC3 ΔHS Stable Cell Line	CHEK-ATP212
HEK293/Human c-MET&ErbB3 Stable Cell Line	CHEK-ATP217
HEK293/Human BCMA Stable Cell Line	CHEK-ATP218