K562/Anti-human CD3 & Anti-human CD16a Stable Cell Line

Catalog No.	Size
CK562-STP348	2 × (1 vial contains ~5×10^6 cells)

• *Description*

This K562-derived target cell line is engineered to display membrane-anchored anti-human CD3 and anti-human CD16 binding modules. The two surface binders recruit and activate CD3-positive T cells and CD16-positive effector cells at the target-cell interface, enabling immune-cell-mediated cytotoxicity to be measured without requiring a matched HLA-peptide pair or expression of a defined tumor-associated antigen.

• *Application*

The product is designed as a standardized, broadly applicable target-cell reagent for functional testing of heterogeneous immune-cell preparations such as tumor-infiltrating lymphocytes (TILs), cytokine-induced killer (CIK) cells, expanded T-cell products, NK-containing cell products, and mixed lymphocyte populations.

• *Cell Line Profile*

Cell line	K562/Anti-human CD3 & Anti-human CD16a Stable Cell Line
Host Cell	K562
Property	Suspension
Selection Marker	NA
Complete Growth Medium	RPMI 1640 + 10% FBS
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- RPMI 1640 Medium (Gibco, Cat. No. 11875-085)
- Fetal bovine serum (ExCell, Cat. No. FSP500)
- Phosphate Buffered Saline (Solarbio, Cat. No. P1020)
- Complete Growth Medium: RPMI 1640 + 10% FBS, 1%P/S
- Freeze Medium: 90% FBS+10% DMSO
- T-25 Culture flask (BIOFIL, Cat. No. TCF012050)
- T-75 Culture flask (BIOFIL, Cat. No. TCF012250)
- Cryogenic storage vials (NEST, Cat. No. 607101)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell800)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

• *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 immediately after thawing, and full recovery (viability >90%) can take up to 1-2 weeks. Once the cell density reaches approximately $1–1.5\times10^6$ viable cells/mL, adjust it to $2 \times 10^5–5 \times 10^5$ viable cells/mL by replacing the existing complete growth medium. Avoid allowing the cell density to exceed 1.5×10^6 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.

• *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.

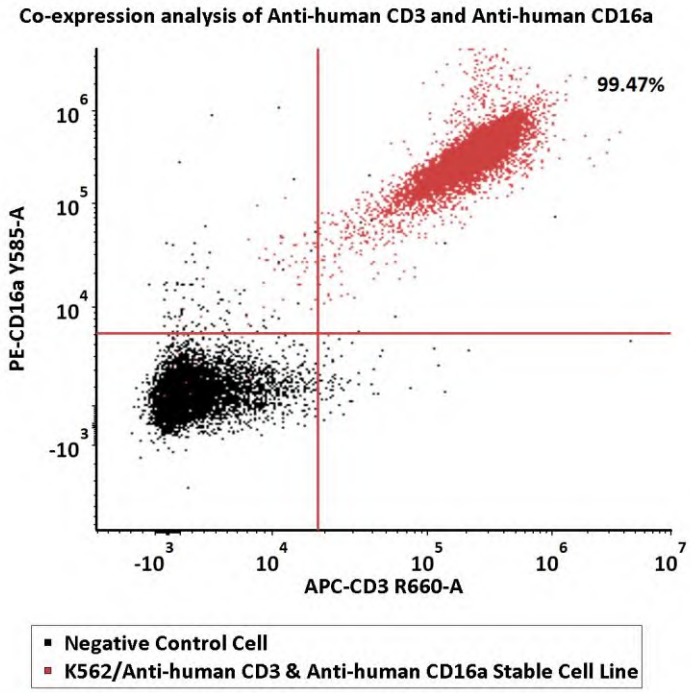
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *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.

K562/Anti-human CD3 & Anti-human CD16a Stable Cell Line Development Service Data Sheet

• *Receptor Assay*



Catalog No.	Stable Cell Line	MFI for Anti-human CD16a (PE)	MFI for Anti-human CD3 (APC)
NA	Negative Control Cell	1545.2	1109.7
CK562-STP348	K562/Anti-human CD3 & Anti-human CD16a Stable Cell Line	219876.7	315697.5

Fig1. Co-expression analysis of anti-human CD3 and anti-human CD16a on K562/Anti-human CD3 & Anti-human CD16a Stable Cell Line by FACS. Cell surface staining was performed on the K562/Anti-human CD3 & Anti-human CD16a Stable Cell Line and negative control cells, with the APC channel used to detect anti-human CD3 and the PE channel used to detect anti-human CD16a.

• *Application*

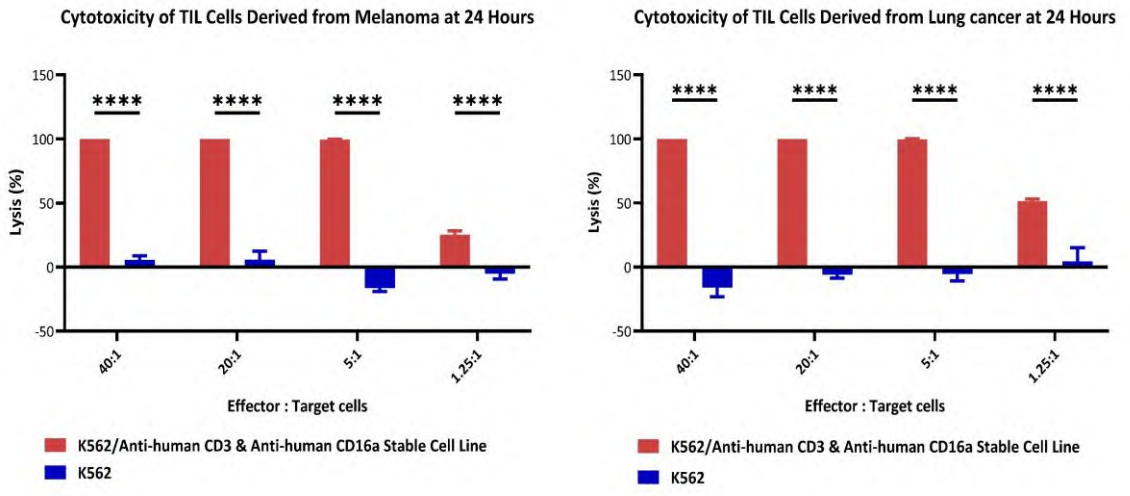


Fig2. The K562/Anti-human CD3 & Anti-human CD16a stable cell line was employed as a target to assess the cytotoxic capacity of tumor-derived TILs. TILs were co-cultured with either parental K562 cells or the K562/Anti-human CD3 & Anti-human CD16a stable cell line, and luciferase activity was quantified as a readout of target cell viability. Across multiple tumor types, TILs exhibited markedly augmented cytolytic activity against the engineered K562 line relative to parental K562 controls.

• *Application*

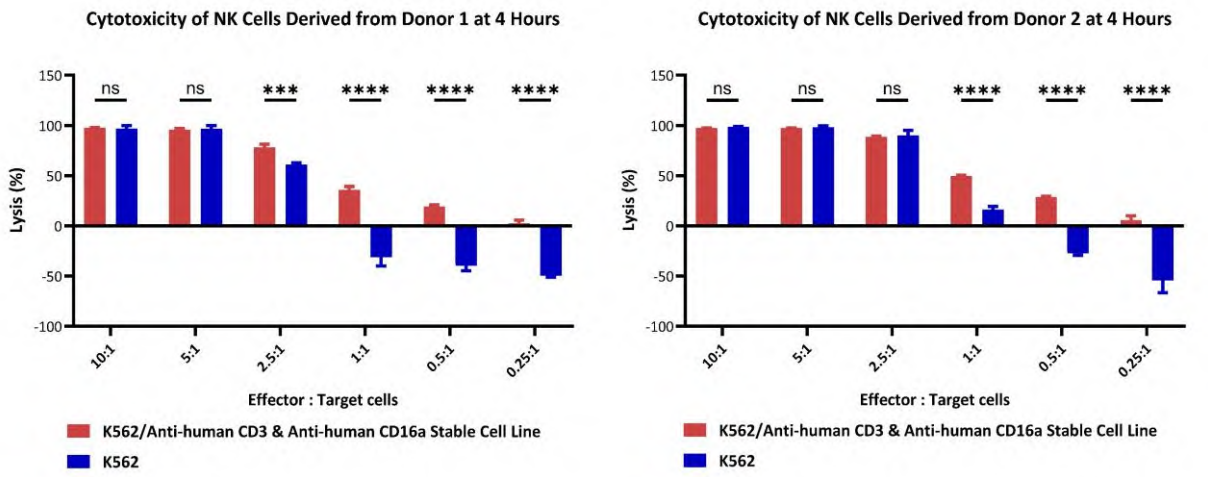


Fig3. The K562/Anti-human CD3 & Anti-human CD16a stable cell line was utilized to evaluate NK cell-mediated cytotoxicity. NK cells were co-cultured with either parental K562 cells or the K562/Anti-human CD3 & Anti-human CD16a stable cell line, followed by quantification of luciferase activity. Although NK cells displayed intrinsic cytotoxicity against K562 cells, lysis of the engineered line was substantially greater than that of the unmodified line at low effector-to-target (E:T) ratios.

- ***Application***

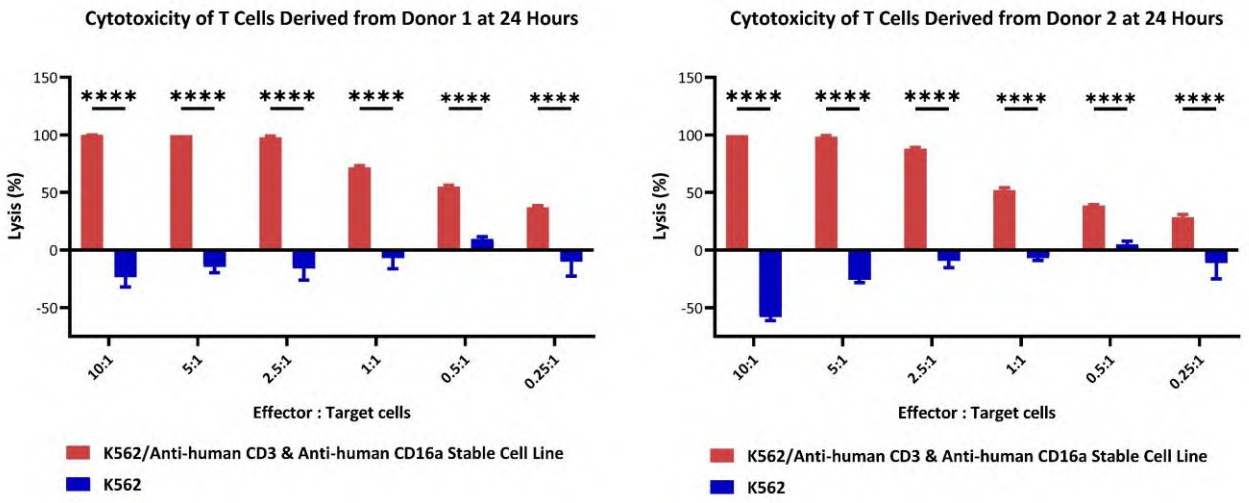


Fig4. The K562/Anti-human CD3 & Anti-human CD16a stable cell line was used to evaluate T cell-mediated cytotoxicity. Donor-derived T cells were co-cultured with either parental K562 cells or the K562/Anti-human CD3 & Anti-human CD16a stable cell line, and luciferase activity was measured thereafter. T cells from multiple healthy donors demonstrated significantly enhanced killing efficacy against parental K562 cells.