

Raji/Human CD19 Knockout Stable Cell Line Development Service

Catalog No.	Size	
SCRAJ-STT216	$2 \times (1 \text{ vial contains } \sim 5 \times 10^6 \text{ cells})$	

• Description

The Raji/Human CD19 Knockout Stable Cell Line was generated from Raji cells by CRISPR/Cas9-mediated knockout of human CD19 (Gene ID:930). The expression level of human CD19 was confirmed by flow cytometry. Mutated sequences of human CD19 produced by non-homologous end joining (NHEJ) were confirmed through genomic sequencing.

• Application

• Useful for cell-based CD19 target-specific analysis

• Cell Line Profile

Cell line	Raji/Human CD19 Knockout Stable Cell Line		
Host Cell	Raji		
Property	suspension		
Complete Growth Medium	RPMI-1640 + 10% FBS		
Selection Marker	NA		
Incubation	37°C with 5% CO ₂		
Doubling Time	16-20 hours		
Transduction Technique	Lentivirus		



• Materials Required for Cell Culture

- RPMI Medium 1640 medium (Gibco, Cat.No. 11875-093)
- Fetal bovine serum (Gibco, Cat.No.10091-148)
- Penicillin-Streptomycin (Gibco, Cat.No.15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat.No.SH30256.01)
- Culture Medium: RPMI Medium 1640 + 10% FBS, 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-II)
- CO₂ Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

• Recovery

- 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 5 minutes).
- 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
- 3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium.
- 4. Count viable cells and spin at approximately 1000 rpm for 5 minutes.
- 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 1×10⁶ viable cells/mL and transfer cells to an appropriate size vessel.
- 6. Incubate at 37°C with 5% CO₂ incubator.



• Subculture

Adjust the cell density at 1×10^5 - 2×10^5 viable cells/mL by the addition of fresh medium or replacement of culture medium. Do not allow the cell density to exceed 2×10^6 cells/mL. T-75 flasks are recommended for subculturing.

• **Medium Renewal:** Add fresh culture medium every 3 to 4 days (depending on cell density)

• Cryopreservation

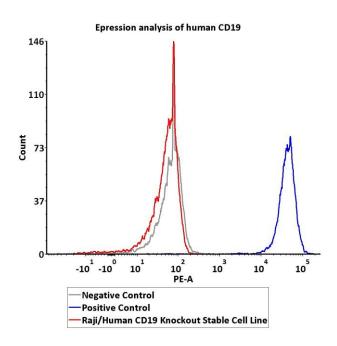
- 1. Count viable cells and harvest the cell suspension.
- 2. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
- 3. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a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

• Storage

- Product format: Frozen
- Storage conditions: Liquid nitrogen immediately upon receipt



• Receptor Assay



Catalog No.	Stable Cell Line	MFI for CD19 (PE)
NA	Negative Control Cell	69.15
NA	Positive Control Cell	41297.73
SCRAJ-STT216	Raji/Human CD19 Knockout Stable Cell Line	55.67

Fig1. Expression analysis of human CD19 on Raji/Human CD19 Knockout Stable Cell Line by FACS.

Cell surface staining was performed on Raji/Human CD19 Knockout Stable Cell Line using PE-labeled anti-human CD19 antibody. The Raji cells were stained with PE-labeled anti-human CD19 antibody as the positive control cell. The Raji cells were stained with PE-labeled isotype control antibody as the negative control cell.



• Sequencing Analysis

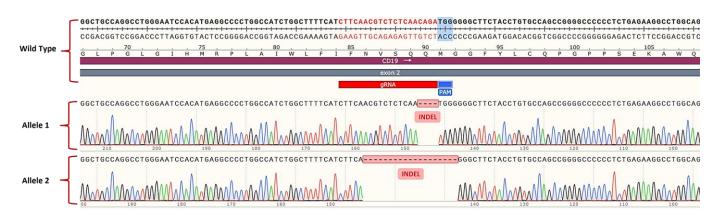


Fig2. Genomic Sequencing of human CD19 in the Raji/Human CD19 Knockout Stable Cell Line.

Sanger sequencing was used for analysis of CRISPR-mediated mutations. The sequencing results demonstrated that the selected sgRNA worked effectively with Cas9 on human CD19 gene in the Raji/Human CD19 Knockout Stable Cell Line.



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• Related Products

<u>Products</u> <u>Cat.No.</u>

Raji/Human PD-L1 Stable Cell Line Development Service Raji/Human CD155 Stable Cell Line Development Service SCRAJ-STT075 SCRAJ-STT076