Raji/Luc & Human CD20 Knockout Stable Cell Line Data Sheet

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Raji/Luc & Human CD20 Knockout Stable Cell Line

Catalog No.	Size
CRAJ-STK285	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Raji/Luc & Human CD20 Knockout Stable Cell Line was generated from Raji cells through targeted knockout of human CD20 (Gene ID: 931) and engineered to express the firefly luciferase reporter. The expression level of human CD20 was confirmed by flow cytometry. The luciferase activity was confirmed by the detection of luminescence signal. Mutated sequences of human CD20 produced by non-homologous end joining (NHEJ) were confirmed through genomic sequencing.

• *Application*

- Useful for cell-based CD20 target-specific analysis
- Used as target cells for luminescence-based cytotoxicity evaluation

• *Cell Line Profile*

Cell line	Raji/Luc & Human CD20 Knockout Stable Cell Line
Host Cell	Raji
Property	suspension
Complete Growth Medium	RPMI Medium 1640 + 10% FBS
Selection Marker	Puromycin (2 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	18-22 hours

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• *Materials Required for Cell Culture*

- RPMI Medium 1640 (ATCC, Cat. No. 30-2001)
- Fetal bovine serum (Gibco, Cat. No. 10091-148)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)

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), 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)

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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% 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 1.5×10^6 viable cells/mL, adjust the density to a range of 1×10^5-2×10^5 viable cells/mL by either adding the fresh culture medium or replacing the existing culture medium. Avoid allowing the cell density to exceed 2×10^6 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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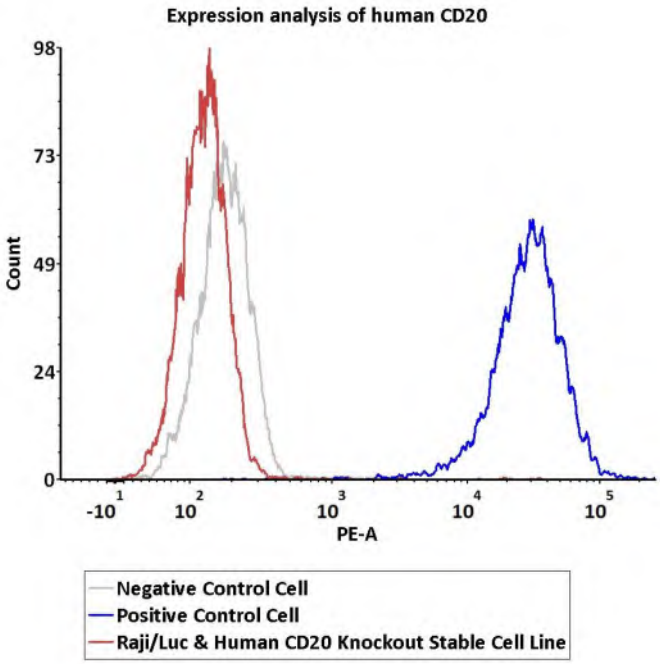
- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for CD20 (PE)
NA	Negative Control Cell	171.07
NA	Positive Control Cell	28337.65
CRAJ-STK285	Raji/Luc & Human CD20 Knockout Stable Cell Line	124.49

Fig1. Expression analysis of human CD20 on Raji/Luc & Human CD20 Knockout Stable Cell Line by FACS. Cell surface staining was performed on Raji/Luc & Human CD20 Knockout Stable Cell Line using PE-labeled anti-human CD20 antibody. The Raji cells were stained with PE-labeled anti-human CD20 antibody as the positive control cell. The Raji cells were stained with PE-labeled isotype control antibody as the negative control cell.

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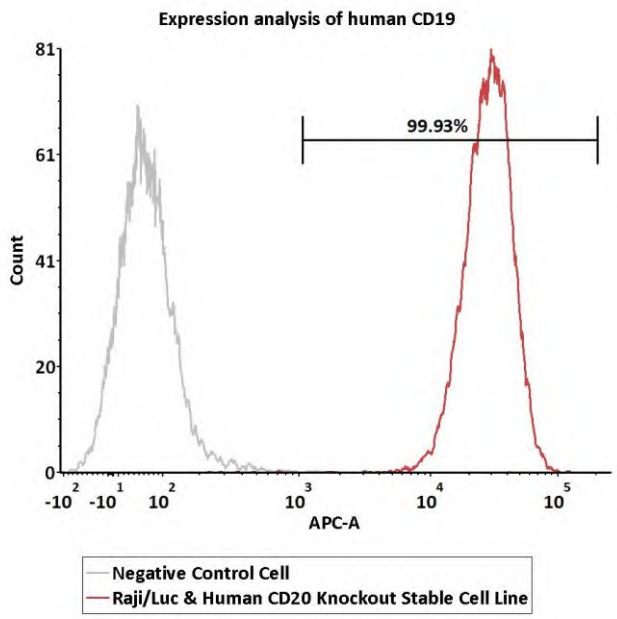
- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for CD19 (APC)
NA	Negative Control Cell	55.83
CRAJ-STK285	Raji/Luc & Human CD20 Knockout Stable Cell Line	27793.48

Fig2. Expression analysis of human CD19 on Raji/Luc & Human CD20 Knockout Stable Cell Line by FACS. Cell surface staining was performed on Raji/Luc & Human CD20 Knockout Stable Cell Line using APC-labeled anti-human CD19 antibody. The Raji/Luc & Human CD20 Knockout Stable Cell Line was stained with APC-labeled isotype control antibody as the negative control cell.

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• *Luminescence Assay*

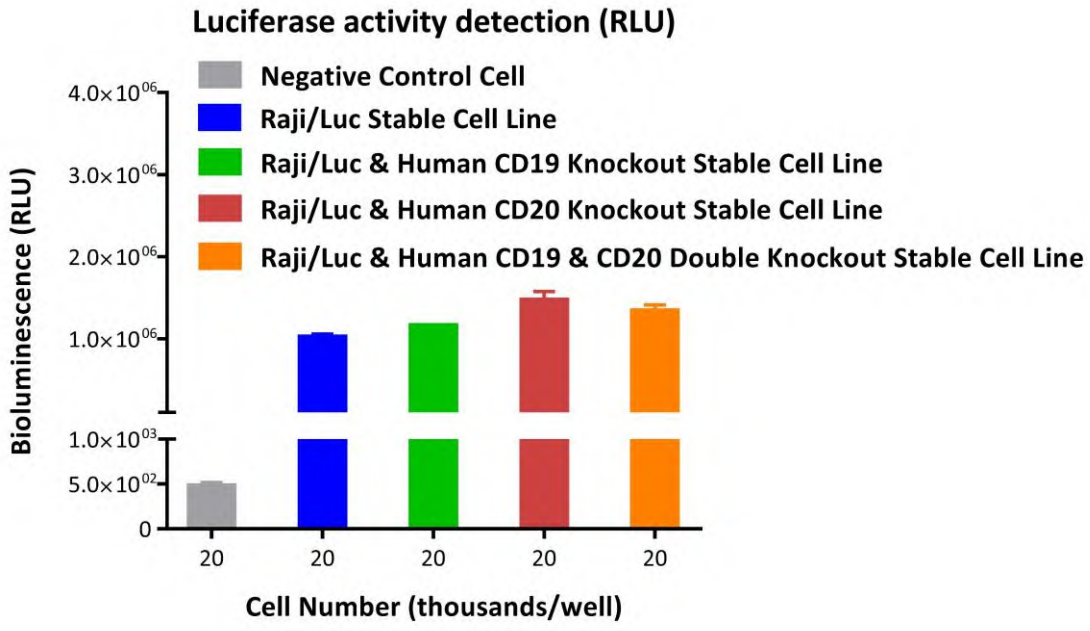

Fig3. Expression analysis of luciferase on Raji/Luc & Human CD20 Knockout Stable Cell Line (RLU). The luminescence signals of Negative Control Cell, Raji/Luc Stable Cell Line (Cat. No. CRAJ-STK287), Raji/Luc & Human CD19 Knockout Stable Cell Line (Cat. No. CRAJ-STK286), Raji/Luc & Human CD20 Knockout Stable Cell Line, and Raji/Luc & Human CD19 & CD20 Double Knockout Stable Cell Line (Cat. No. CRAJ-STK284) were all detected simultaneously by incubating with the luciferase substrate. The RLU value of Raji/Luc & Human CD20 Knockout Stable Cell Line was approximately 1.47×10^6 at the density of 2×10^4 cells/well.

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• *Luminescence Assay*

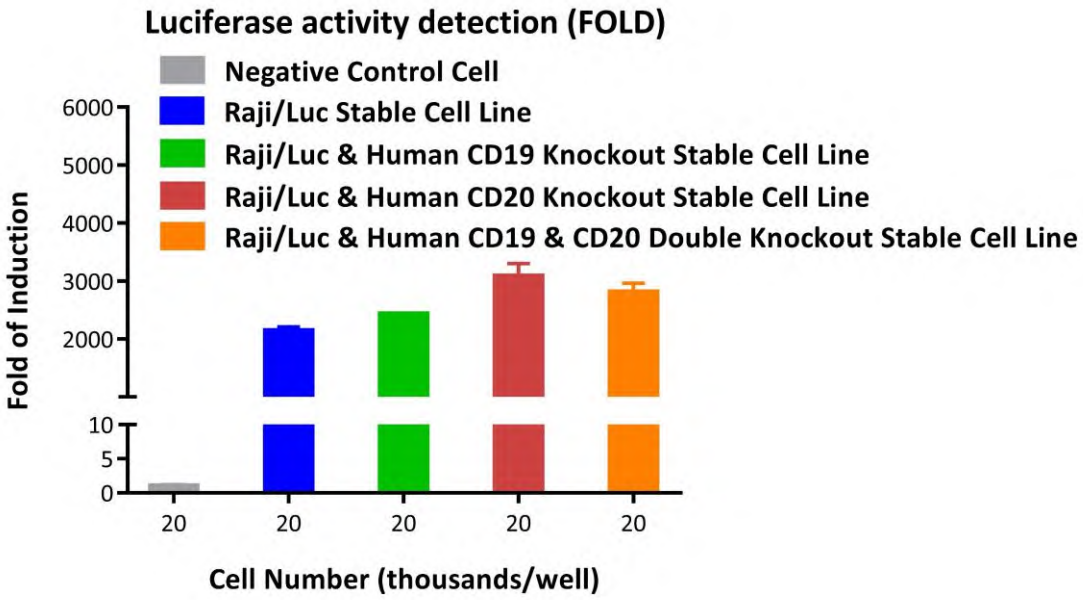

Fig4. Expression analysis of luciferase on Raji/Luc & Human CD20 Knockout Stable Cell Line (FOLD). The luminescence signals of Negative Control Cell, Raji/Luc Stable Cell Line (Cat. No. CRAJ-STK287), Raji/Luc & Human CD19 Knockout Stable Cell Line (Cat. No. CRAJ-STK286), Raji/Luc & Human CD20 Knockout Stable Cell Line, and Raji/Luc & Human CD19 & CD20 Double Knockout Stable Cell Line (Cat. No. CRAJ-STK284) were all detected simultaneously by incubating with the luciferase substrate. The max induction fold of Raji/Luc & Human CD20 Knockout Stable Cell Line was approximately 3084.24 at the density of 2×10^4 cells/well.

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• *Sequencing Analysis*

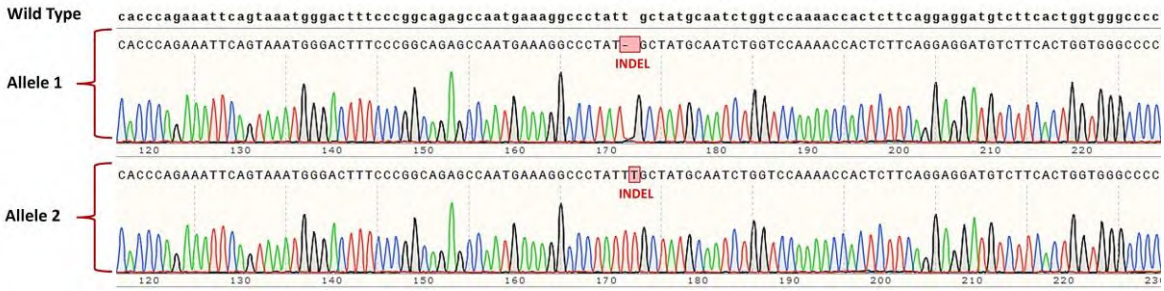

Fig5. Genomic Sequencing of human CD20 in the Raji/Luc & Human CD20 Knockout Stable Cell Line. Sanger sequencing was used for mutation analysis of human CD20. The sequencing results demonstrated that frameshift mutations were generated in the human CD20 gene in the Raji/Luc & Human CD20 Knockout Stable Cell Line.

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• *Related Products*

Products	Cat. No.
Raji/Luc & Human CD19 Knockout Stable Cell Line	CRAJ-STK286
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
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+)	CJUR-STF247
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+)	CJUR-STK269
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+ CD8+)	CJUR-STK270
Jurkat/Human TRBV9 Stable Cell Line	CJUR-STP293
Jurkat/Human TRBV9 & Luc Stable Cell Line	CJUR-STP292
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
HEK293/Human Claudin-1 Stable Cell Line	CHEK-ATP124

• *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
HEK293/Human LY6G6D Stable Cell Line	CHEK-ATP137
HEK293/Human Claudin-6 Stable Cell Line	CHEK-ATP138
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
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

• *Related Products*

Products	Cat. No.
CHO/Human CDCP1 (R368A, K369A) Stable Cell Line	CCHO-ATP234
CHO/Human CDCP1 (NTF&CTF) Stable Cell Line	CCHO-ATP235
HEK293/Human Tissue Factor Stable Cell Line	CHEK-ATP240
CHO/Human PSMA Stable Cell Line	CCHO-ATP246
CHO/Human Integrin alpha V beta 6 Stable Cell Line	CCHO-ATP254
HEK293/Human CD133 Stable Cell Line	CHEK-ATP255
Jurkat/Luc Stable Cell Line	CJUR-STP258
HEK293/Human FGF R2 (IIIb) Stable Cell Line	CHEK-ATP259
HEK293/Human STEAP2 Stable Cell Line	CHEK-ATP263
CHO/Human PD-1 Stable Cell Line	CCHO-ATP266