Technical Bulletin

Raji CD19 Antigen Panel Cell Lines

Catalog Number ATG001

Product Description

The Raji CD19 antigen panel is a series of three (3) genetically modified cell lines that targets the CD19 locus in Raji B-cells.

The panel consists of the following cell lines (Figure 1):

1. ATG001A-1VL: Raji CD19 Knockout
2. ATG001B-1VL: Raji CD19 Low
3. ATG001C-1VL: Raji CD19 High

CompoZr® zinc finger nuclease (ZFN) technology was used to create a pan-allelic, targeted knockout (KO) of the CD19 gene in wild type Raji cells (Catalog Number 85011429-1VL). Generation of the Raji CD19 KO cell line was confirmed via next-generation sequencing (NGS) analysis and flow cytometry as shown in Figure 2.

Following single cell cloning and expansion of the Raji CD19 KO cell line, MISSION® lentiviral particles were used to randomly integrate and express exogenous, full-length CD19 cDNA at low or high levels relative to the knockout to generate the Raji CD19 Low (ATG001B-1VL) and Raji CD19 High (ATG001C-1VL) cell lines.

CD19 protein expression was measured in the antigen panel via fluorescent-activated cell sorting (FACS) as shown in Figure 3.

Figure 1
Generation of the Tumor-Associated Antigen Panel Cell Lines

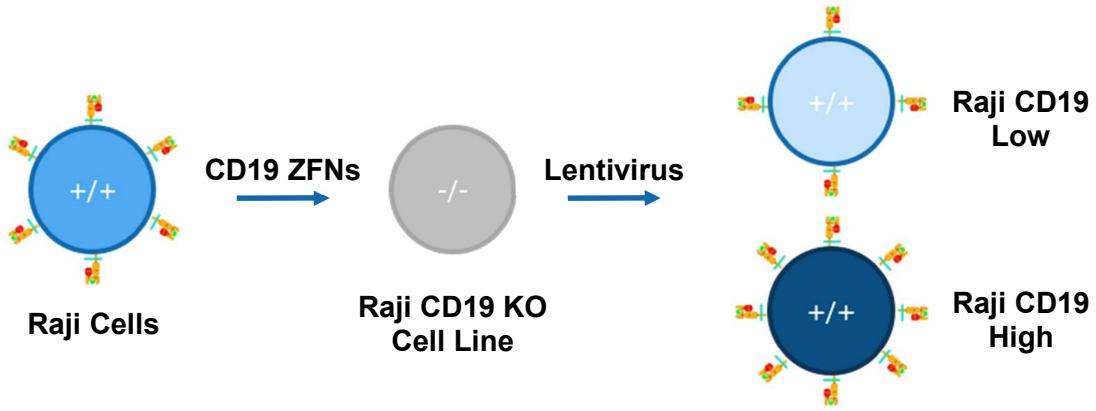
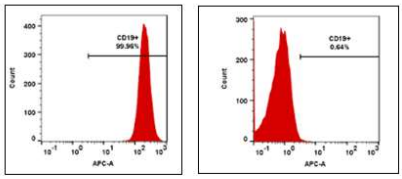


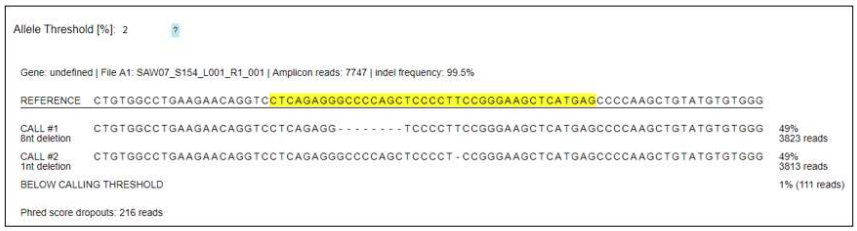
Figure 2
Genomic mutations in the CD19 locus.
Site-specific pan-allelic -8 and -1 deletions at the CD19 locus in Raji cells

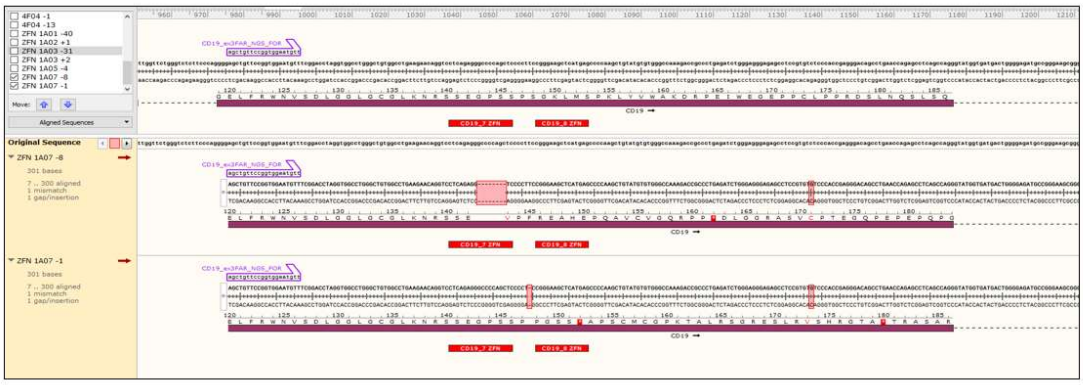
Top panel: Left: wild-type Raji cells and Raji KO cells stained with anti-CD19. Right: NGS sequencing results, ZFN target site is highlighted in yellow.
Bottom panel: Raji CD19 deletion allele sequence alignment results showing 1 and 8 base pair deletions and premature stop codon locations.



WT Raji cells stained with APC-CD19 antibody

CD19KO Raji cells stained with APC-CD19 antibody





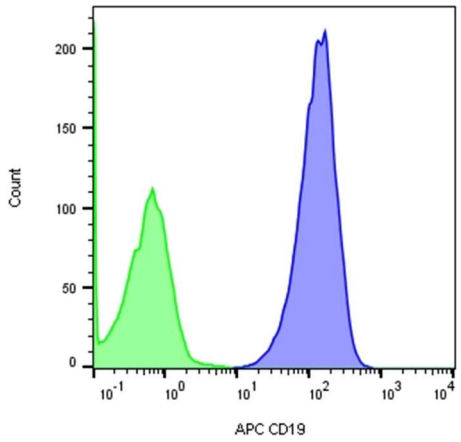
Genomic sequence at the target region recognized by the ZFN pair.
CTCAGAGGGCCCCCAGCTCCCCTTCCGGGAAGCTCATGAG

NGS PCR for knockout
Forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNggctatgaggaacctgacagtg
Reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNtcatcctcagggttctcgtagc

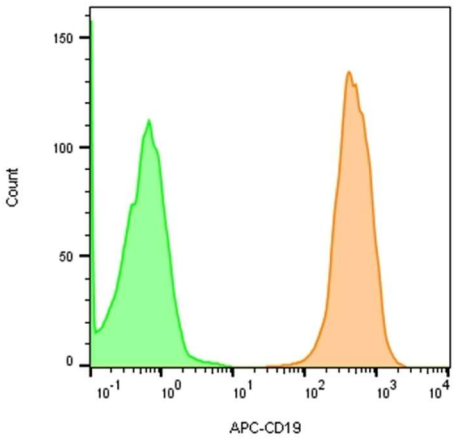
Figure 3

CD19 Expression in the Raji CD19 Antigen Panel

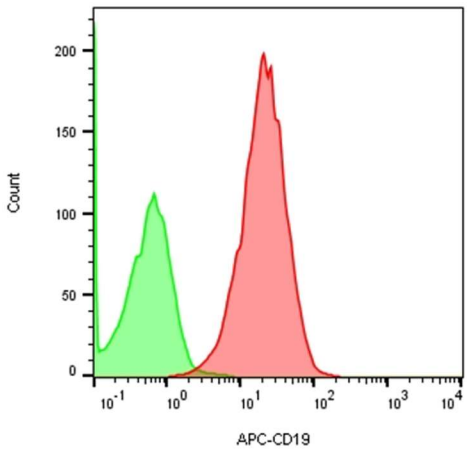
Raji cells were stained with an APC-conjugated CD19 antibody and analyzed by MacsQuant. Cells were gated by scatter, doublets were excluded, and only live cells were analyzed. Appropriate IgG controls were used and did not stain any of the cells (data not shown).



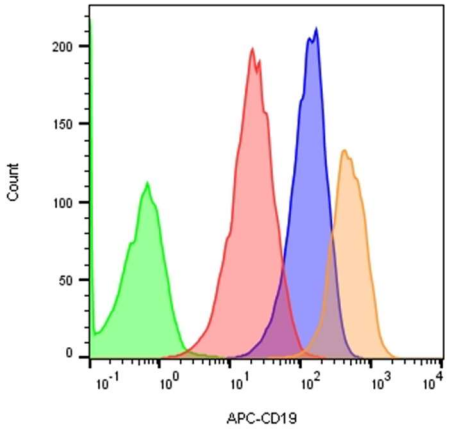
Raji CD19 KO cells (green) compared to wild type Raji cells (blue).



Raji CD19 High cells (orange) compared to Raji CD19 KO cells (green).



Raji CD19 Low cells (green) compared to Raji CD19 KO cells (red).



CD19 expression in the Raji CD19 antigen panel cell lines compared to wild type Raji cells. KO (green), low (red), high (orange), wild type Raji cells (blue).

Components

This product is three (3) cryovials containing a minimum of 1 million Raji cells in each vial.

The cryoprotectant medium used is CryoStor® cell cryopreservation medium containing 10% DMSO (Catalog Number C2874).

Cell Line Description

Organism: Homo sapiens (human)
Tissue: Lymph
Gender: Male
Morphology: Lymphoblastoid
Growth Properties: Suspension

DNA Profile

STR-PCR Data:
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 13
D16S539: 8,11
D5S818: 10,13
D7S820: 10
THO1: 6,7
TPOX: 8,13
vWA: 16,19

The STR profile of this cell line matches that of its parental cell line European Collection of Authenticated Cell Cultures (ECACC) Catalog Number 85011429. Please see the ECACC Catalog Number 85011429 datasheet for additional information about the origin of this cell line.

Reagents and Equipment Required but Not Provided

- RPMI-1640 Medium with L-glutamine and sodium bicarbonate, Catalog Number R8758
- Fetal Bovine Serum, USA origin, sterile-filtered, Catalog Number F2442
- Biological safety cabinet
- 70% ethanol (prepared from Ethanol, Catalog Number E7148)
- Bio-Pure™ alcohol wipes, Catalog Number Z688487
- 37 °C water bath (operating range 35-38 °C)
- Sterile 15 mL conical tubes
- Centrifuge
- Serological pipettor with 1, 2, 5, 10, and 25 mL sterile pipettes
- Vacuum aspiration system and sterile plastic or glass aspiration tips
- Sterile culture flasks or plates for suspension cells
- 37 °C, 5% CO_2 incubator

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Precaution: It is recommended that protective gloves and clothing always be used, and a full-face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris.

Storage/Stability

Store cells at -196 °C (liquid nitrogen)

Upon receiving a shipment of frozen cells, it is important the end user gives the shipment attention without delay. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70 °C. Storage at -70 °C will result in loss of viability.

At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Procedure

Medium Preparation Instructions

The base medium for this cell line is RPMI 1640 with L-Glutamine (Catalog Number R8758).

Complete Medium: To make the complete growth medium, add Fetal Bovine Serum (Catalog Number F2442) to a final concentration of 10%.

Thawing of Frozen Cells

1. Thaw the vial by gentle agitation in a 37 °C water bath for ~1 minute. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
2. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol solution. All the operations from this point on should be carried out under aseptic conditions.
3. Transfer the cell suspension to a 15 ml conical tube containing 9 mL of warmed Complete Medium.
4. Centrifuge the cells at 125 × g for 5-7 minutes at room temperature.
5. Aspirate the media from the tube. Resuspend the cell pellet with 4 mL of warmed Complete Medium and plate into a 25 cm^2 culture flask or appropriately sized vessel.
6. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested, prior to the addition of the vial contents, the culture vessel containing the Complete Medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6) and temperature (37 °C).
7. Incubate cultures at 37 °C in an incubator containing an atmosphere of 5% CO_2 in air.

Sub-culturing Procedure

Cells should be sub-cultured when at 0.9 – 1.1 × 10^6 cells/mL.

1. Perform cell count and dilute with fresh media to a cell concentration of 400,000 cells/mL.
2. Add appropriate aliquots of the cell suspension into new culture vessels.
3. Incubate cultures at 37 °C in an incubator containing an atmosphere of 5% CO_2 in air.

References

1. Giard, D.J., et al. *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors., *J. Natl. Cancer Inst.*, **51(5),** 1417-23 (1973). PMID: 4357758

Additional product and technical information can be obtained by searching for the catalog number at sigmaaldrich.com.

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Tumor-Associated Antigen Panel Cell Lines

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