Technical Bulletin

Raji HLA Panel Cell Lines

Catalog Number HLA001

Product Description

The Raji HLA panel is comprised of nine (9) genetically modified cell lines targeting MHC Class I HLA molecules in a Raji lymphoblast parental cell line background. The full HLA panel consists of a Beta-2 microglobulin (β2M) knockout cell line and an additional eight (8) monoallelic HLA expression cell lines.

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

1. HLA001A-1VL: Raji B2M KO Cells
2. HLA001B-1VL: Raji HLA-A*02:01 Cells
3. HLA001C-1VL: Raji HLA-A*01:01 Cells
4. HLA001D-1VL: Raji HLA-A*03:01 Cells
5. HLA001E-1VL: Raji HLA-A*11:01 Cells
6. HLA001F-1VL: Raji HLA-A*24:02 Cells
7. HLA001G-1VL: Raji HLA-B*15:10 Cells
8. HLA001H-1VL: Raji HLA-B*07:02 Cells
9. HLA001K-1VL: Raji HLA-B*40:01 Cells

CompoZr® zinc finger nuclease (ZFN) technology was used to create a targeted knockout (KO) of the Beta-2 microglobulin (β2M) gene in wild type Raji cells (Catalog Number 85011429). Generation of the Raji β2M KO cell line was confirmed to not express endogenous cell surface MHC Class I HLA molecules (HLA-A or HLA-B) via next-generation sequencing (NGS) analysis as shown in Figure 2 and Figure 3 and fluorescence-activated cell sorting (FACS) as shown in Figure 4.

Following single cell cloning and expansion of the Raji β2M KO cell line, MISSION® lentiviral particles were used to generate the eight (8) monoallelic HLA cell lines which express individual HLA-A or HLA-B subtypes on the cell surface via a β2M:HLA fusion protein.

Individual HLA-A or HLA-B subtype expression was measured in the HLA panel via fluorescent-activated cell sorting (FACS) as shown in Figure 4.

Figure 1
Generation of the HLA Panel Cell Lines

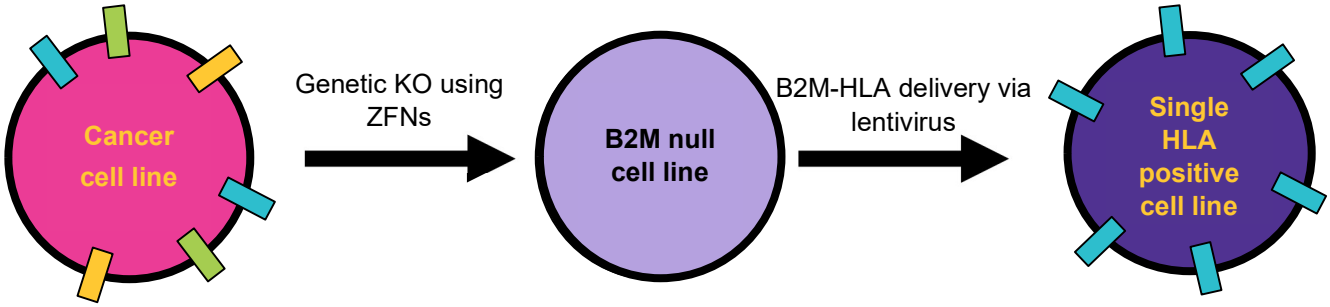


Figure 2. Genomic mutations in the β2M locus. -4 and -1 bp deletions within exon 2 of β2M in Raji cells. Reference sequence in wild type Raji cells, ZFN target site is highlighted in yellow.

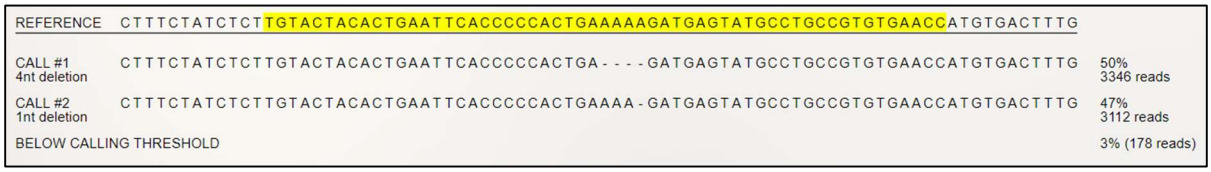
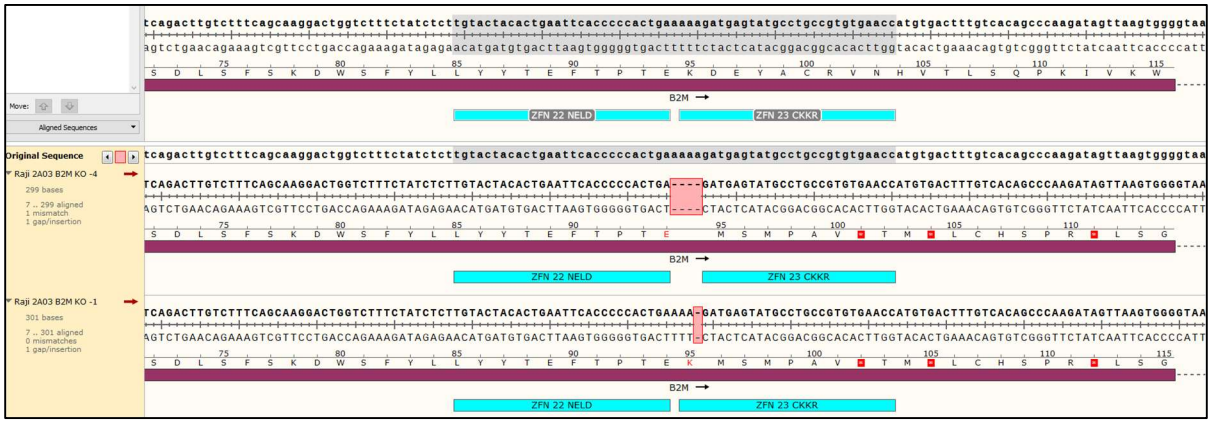


Figure 3. Exonic alignment of -4 and -1 bp deletions. Both deletions result in predicted premature stop codons (denoted with red asterisks) in exon 2 of β2M.



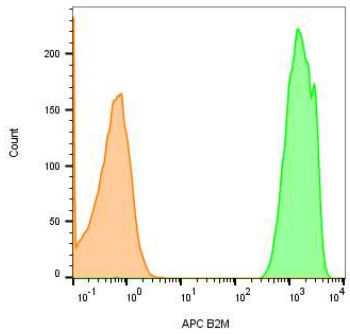
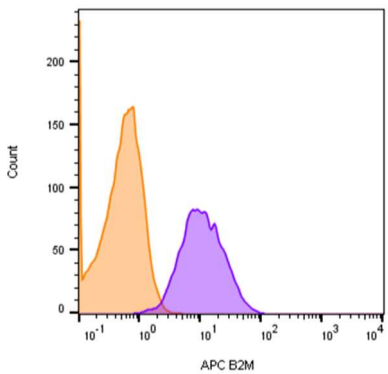
Genomic sequence at the target region recognized by the ZFN pair.
TGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTGCCGTGTGAACC

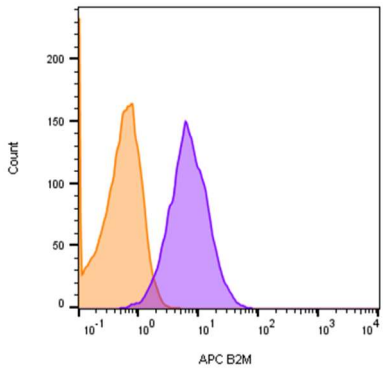
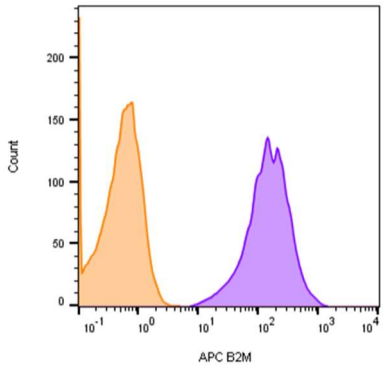
NGS PCR for genotyping
Forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNctgggtttcatccatccgaca
Reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNtgggatgggactcattcagg

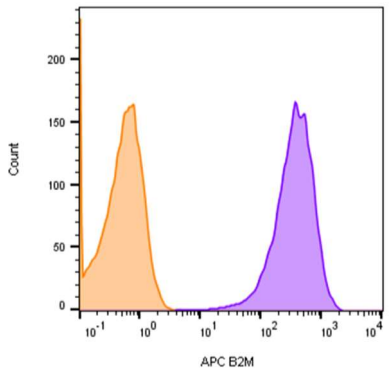
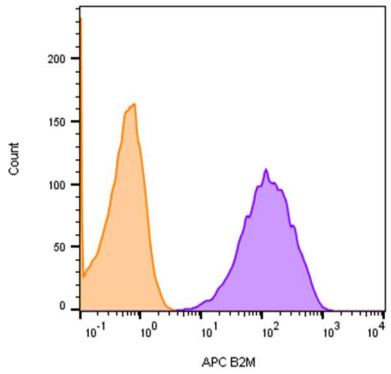
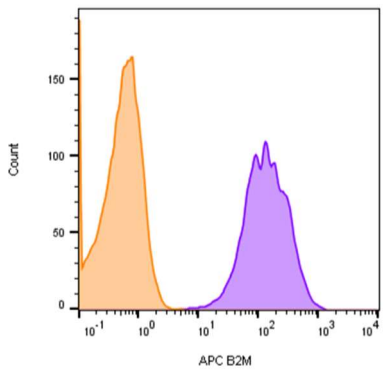
Wildtype amplicon sequence (321 bp)

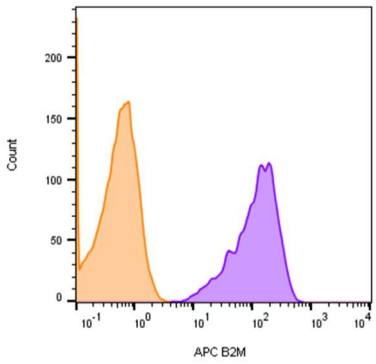
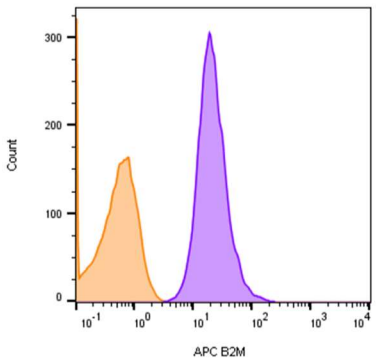
CTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATT
CAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGA
TGAGTATGCCTGCCGTGTGAACCATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGGTAAGTCTTACAT
TCTTTTGTAAGCTGCTGAAAGTTGTGTATGAGTAGTCATATCATAAAGCTGCTTTGATATAAAAAAGGTCTAT
GGCCATACTACCCTGAATGAGTCCCATCCCA

Figure 4. Confirmation of β2M KO and expression of individual HLA-A or HLA-B subtypes in the Raji HLA Panel via FACS Analysis.

Product	FACS Results
HLA001A-1VL Raji B2M KO Cells	 β2M protein expression in Raji WT cells (green) compared to expression in Raji β2M KO cells (orange).
HLA001B-1VL Raji HLA-A*02:01 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-A*02:01 cells

Product	FACS Results
HLA001C-1VL Raji HLA-A*01:01 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-A*01:01 cells
HLA001D-1VL Raji HLA-A*03:01 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-A*03:01 cells

Product	FACS Results
HLA001E - 1VL Raji HLA-A*11:01 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-A*11:01 cells
HLA001F - 1VL Raji HLA-A*24:02 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-A*24:02 cells
HLA001G - 1VL Raji HLA-B*15:10 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-B*15:10 cells

Product	FACS Results
HLA001H -1VL Raji HLA-B*07:02 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-B*07:02 cells
HLA001K -1VL Raji HLA-B*40:01 Cells	 Orange: Raji B2M KO cells Purple: Raji HLA-B*40:01 cells

Components

This product is nine (9) 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
D18S51: 17
D21S11:28, 31
D3S1358: 15, 16
D5S818: 10,13
D7S820: 10
D8S1179: 14, 15
FGA: 19, 27
Penta_D: 3.2, 9
Penta_E: 5, 13
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 25 cm^2 or 75 cm^2 culture flasks
- 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 6 mL of warmed Complete Medium and plate into a 25 cm^2 or 75 cm^2 culture flask.
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 the culture at 37 °C in a 5% CO_2 in air atmosphere incubator.

Sub-culturing Procedure

Cells should be sub-cultured when at 0.9 – 1.1 $\times 10^6$ 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
2. Gornalusse, G., Hirata, R., Funk, S. et al., HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat. Biotechnol.* **35**, 765–772 (2017). PMID: 28504668

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

These products are covered by the Purchase Agreement as described in Exhibit 1.

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HLA Panel Cell Lines

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