Technical Bulletin

Jurkat HLA Panel Cell Lines

Catalog Number HLA002

Product Description

The Jurkat HLA panel is comprised of eleven (11) genetically modified cell lines targeting MHC Class I HLA molecules in a Jurkat T lymphocyte parental cell line background. The full HLA panel consists of a Beta-2 microglobulin (β2M) knockout cell line and an additional ten (10) monoallelic HLA expression cell lines.

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

1. HLA002A-1VL: Jurkat B2M KO Cells
2. HLA002B-1VL: Jurkat HLA-A*02:01 Cells
3. HLA002C-1VL: Jurkat HLA-A*01:01 Cells
4. HLA002D-1VL: Jurkat HLA-A*03:01 Cells
5. HLA002E-1VL: Jurkat HLA-A*11:01 Cells
6. HLA002F-1VL: Jurkat HLA-A*24:02 Cells
7. HLA002G-1VL: Jurkat HLA-B*15:10 Cells
8. HLA002H-1VL: Jurkat HLA-B*07:02 Cells
9. HLA002I-1VL: Jurkat HLA-B*08:01 Cells
10. HLA002J-1VL: Jurkat HLA-B*35:01 Cells
11. HLA002K-1VL: Jurkat 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 Jurkat cells (Catalog Number 88042803). Generation of the Jurkat 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 Jurkat β2M KO cell line, MISSION® lentiviral particles were used to generate the ten (10) 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

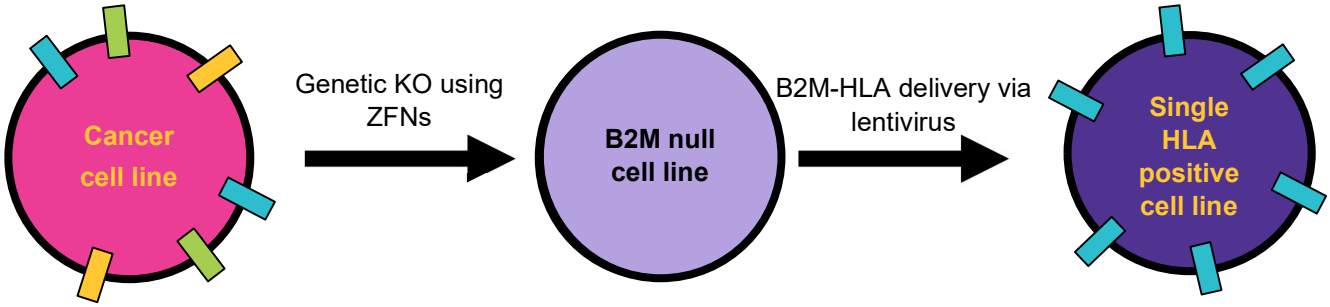


Figure 2. Genomic mutations in the β2M locus. -5 and -1 bp deletions within exon 2 of β2M in Jurkat cells. Reference sequence in wild type Jurkat cells, ZFN target site is highlighted in yellow.

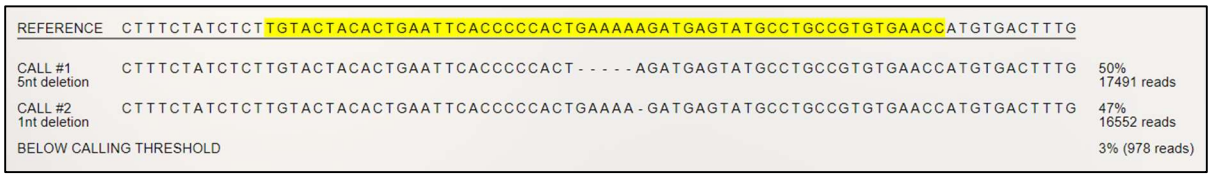


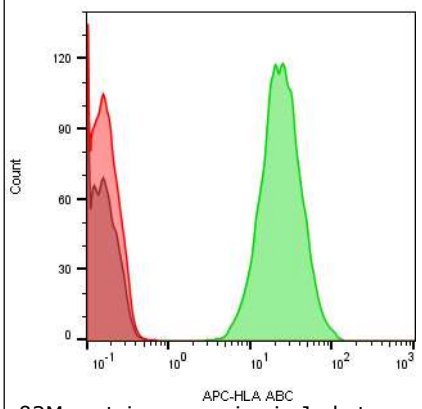
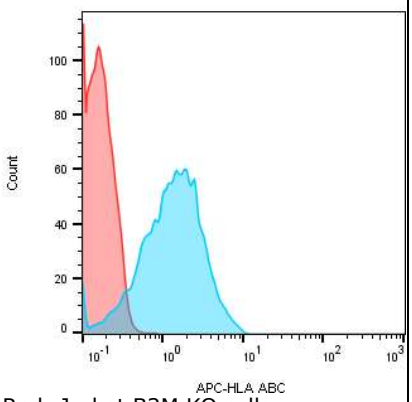
Figure 3. Exonic alignment -5 and -1 bp deletions. Both deletions result in predicted premature stop codons in exon 2 of β2M (indicated by red asterisks). ZFN target sites are shown in cyan.

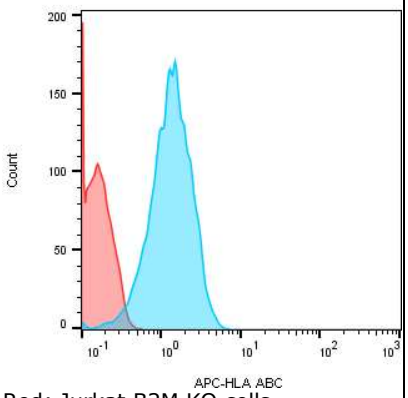
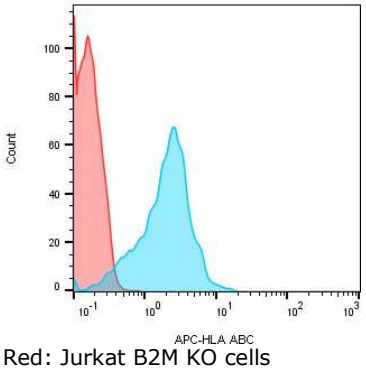
Genomic sequence at the target region recognized by the ZFN pair.
TGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTGCCGTGTGAACC

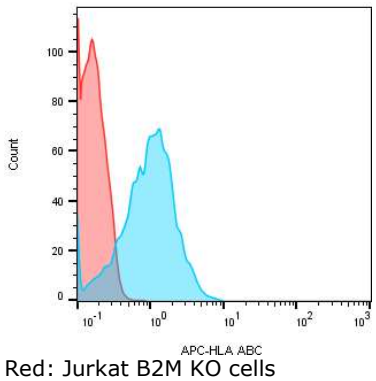
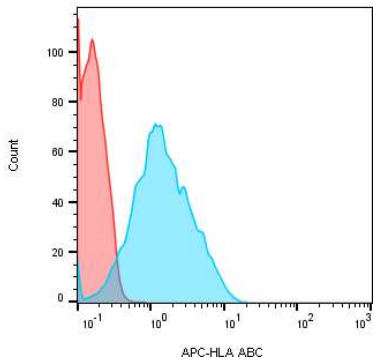
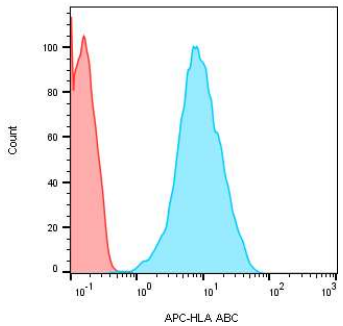
NGS PCR for genotyping
Forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNctgggtttcatccatccgaca
Reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNtgggatgggactcattcagg

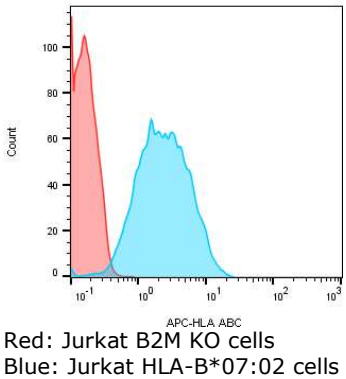
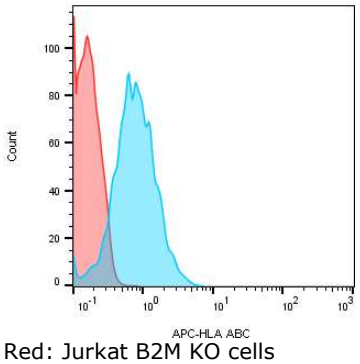
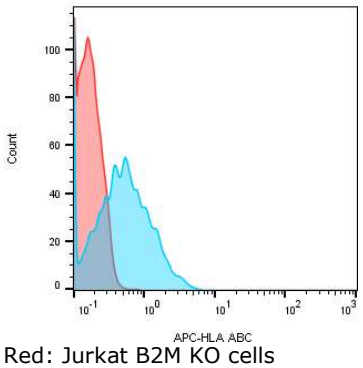
Wildtype amplicon sequence (321 bp)
CTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATT
CAGACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGA
TGAGTATGCCTGCCGTGTGAACCATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGGTAAGTCTTACAT
TCTTTTGTAAGCTGCTGAAAGTTGTGTATGAGTAGTCATATCATAAAGCTGCTTTGATATAAAAAAGGTCTAT
GGCCATACTACCCTGAATGAGTCCCATCCCA

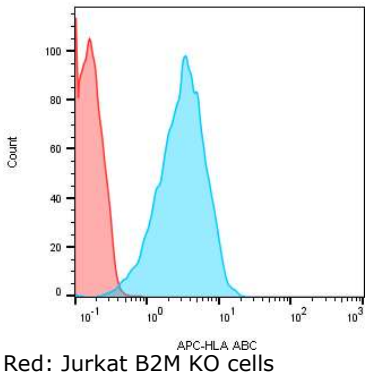
Figure 4. Confirmation of β2M KO and expression of individual HLA-A or HLA-B subtypes in the Jurkat HLA Panel via FACS Analysis. Cells were stained with APC-IgG or APC-HLA ABC antibody (clone W6/32).

Product	FACS Results
HLA002A-1VL Jurkat B2M KO Cells	 β2M protein expression in Jurkat WT cells (green) compared to expression in Jurkat β2M KO cells (red) and parental Jurkat WT stained with an IgG control (gray).
HLA002B-1VL Jurkat HLA-A*02:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-A*02:01 cells

Product	FACS Results
HLA002C-1VL Jurkat HLA-A*01:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-A*01:01 cells
HLA002D-1VL: Jurkat HLA-A*03:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-A*03:01 cells

Product	FACS Results
HLA002E-1VL: Jurkat HLA-A*11:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-A*11:01 cells
HLA002F-1VL: Jurkat HLA-A*24:02 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-A*24:02 cells
HLA002G-1VL: Jurkat HLA-B*15:10 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-B*15:10 cells

Product	FACS Results
HLA002H-1VL: Jurkat HLA-B*07:02 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-B*07:02 cells
HLA002I-1VL: Jurkat HLA-B*08:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-B*08:01 cells
HLA002J-1VL: Jurkat HLA-B*35:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-B*35:01 cells

Product	FACS Results
HLA002K-1VL: Jurkat HLA-B*40:01 Cells	 Red: Jurkat B2M KO cells Blue: Jurkat HLA-B*40:01 cells

Components

This product is eleven (11) cryovials containing a minimum of 1 million Jurkat 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: Blood (leukemic T-cell lymphoblast)
Gender: Male
Morphology: Lymphoblastoid
Growth Properties: Suspension

DNA Profile

STR-PCR Data:
Amelogenin: X,Y
CSF1PO: 12
D13S317: 8, 11
D16S539: 11
D18S51: 13, 22
D21S11: 31.2, 33.2
D3S1358: 16
D5S818: 9
D7S820: 8, 12
D8S1179: 13, 14
FGA: 20
Penta_D: 11, 13
Penta_E: 9, 12
THO1: 6, 9.3
TPOX: 8, 10
vWA: 18, 19

The STR profile of this cell line matches that of its parental cell line European Collection of Authenticated Cell Cultures (ECACC) Catalog Number 88042803. Please see the ECACC Catalog Number 88042803 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 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
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.

Exhibit 1 – Purchase Agreement and Technology Label Licenses

HLA Panel Cell Lines

MISSION® RNAi Licenses - Lentiviral Licenses

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot disclose information, sell or otherwise transfer this product; its components or materials made using this product or its components for any commercial purposes.

This product is licensed under U.S. Pat. Nos. 6,682,907, 8,093,042, 8,512,994 and foreign equivalents from Institut Pasteur, Paris, France and is provided for internal research purposes only. Use of this product or technology for any commercial production or sale of lentiviral tools or products requires a license from Sigma-Aldrich Co. LLC. 3050 Spruce Street, St. Louis, MO 63103, USA. Sigma-Aldrich Co. LLC has the exclusive worldwide rights for licensing the DNA Flap technology covered under the above patents. Use of this product or technology for any commercial development for therapeutic application requires a license from Institute Pasteur, 25-28 Rue du Dr. Roux, 75015 Paris, France.

Zinc Finger Nuclease (ZFN) License Agreement

This Product and its use are the subject of one or more of the following patents controlled by Sangamo BioSciences, Inc.: U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, 6,479,626, US20030232410, US20090203140 and corresponding foreign patent applications and patents.

BEFORE OPENING OR USING THIS PRODUCT, PLEASE READ THE TERMS AND CONDITIONS SET FORTH IN THIS LICENSE AGREEMENT. YOUR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THESE TERMS AND CONDITIONS. If you do not agree to use this Product pursuant to the terms and conditions set out in this License Agreement, please contact Sigma Technical Services within ten days of receipt to return the unused and unopened Product for a full refund; provided, however, that custom-made Products may not be returned for a refund.

The purchase of this Product conveys to you, the buyer, the non-transferable right to use the purchased Product for Licensed Research Use (see definition below) subject to the conditions set out in this License Agreement. If you wish to use this Product for any purpose other than Licensed Research Use, you must first obtain an appropriate license (see information set out below).

This Product may not be used for any purpose other than Licensed Research Use. Your right to use this Product for Licensed Research Use is subject to the following conditions and restrictions:

1. "Licensed Research Use" means any use for research purposes, other than:

(a) Licensing, selling, distributing, or otherwise providing Modified Animals to any third party other than Sigma and its affiliates as provided herein: provided however, that you may provide Modified Animals to researchers within your research organization located at the same research facility or campus. A "Modified Animal" means an animal having a genomic modification at the target site that results from Customer's use of the Product. Modified Animal includes but is not limited to (a) heterozygotes and mosaic animals, (b) the descendants of Modified Animals, (c) animals created from the breeding of Modified Animals with other animals, and (d) animals created by the Customer which contain and/or incorporate genetic information derived from Modified Animals.

(b) GMP production of therapeutic, diagnostic, prophylactic or other medicinal Products intended for use in humans or non-human animals, or any other industrial use solely to the extent involving commercial sale of a Product or service. If a molecule or any derivative of such molecule is used in or administered to humans, then the production of such molecule shall be deemed to be GMP production and therefore in violation of this License Agreement;

(c) use for gene targeting and/or gene regulation to modify the genome of a plant cell, plant, or plant cell culture (in each case, whether constituting or derived from a vascular or non-vascular plant), or alter the nucleic acid or protein expression in a plant cell, plant, or plant cell culture. "Non-vascular" plants shall include but not be limited to algae, moss, and fungi; and

(d) modification or reverse-engineering of the Product in any way or creating any derivatives or sequence variants thereof.

2. You may not transfer the Product, its components, or any materials made through the use of this Product, including Modified Animals, to any third party without prior written approval of Sigma and without the transferee entering into a material transfer agreement with Sigma. Notwithstanding the foregoing:

(a) the Product or materials made through use of the Product may be transferred by you to your legal affiliates or bonafide third party contractors performing paid work on your behalf, with the exception of creation of Modified Animals, provided the use by such third party contractors is limited to performance of work for you; and

(b) you may donate Mice that are Modified Animals as defined above ("Modified Mice") to The Jackson Laboratory, a licensed distributor of Modified Mice.

3. You may not transfer the Product or materials made through use of the Product to third party contractors performing paid work on your behalf for the purposes of creation of Modified Animals.

4. Your right to use the Product will terminate immediately if you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, Modified Animals, and components thereof in your control, and notify Sigma of such in writing.

5. You may not use the Product to support the filing of a patent application in any country in the world that contains claims directed to the Product or its uses.

For information on purchasing a license to this Product for purposes other than Licensed Research Use, contact your local Sigma Sales representative, who will refer you to the proper licensing representative, or in the USA call 800-325-3010.

For information on donating Modified Mice to The Jackson Laboratory, please visit their website at: http://www.jax.org/grc/index.html

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.