SZABO SCANDIC

Part of Europa Biosite

# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


**SZABO-SCANDIC HandelsgmbH**

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

**Description**

The HLA-A/B/C Knockout Electroporation Kit is suitable for cell line and primary T cell engineering via electroporation. The kit contains both the Cas9 enzyme (*Streptococcus pyogenes*) and the gRNA targeting HLA-A/B/C (Human Leukocyte Antigens). This kit is sufficient to engineer up to 5 million primary T cells.

**Background**

HLA (Human Leukocyte Antigens)-A, B, and C, are the three major types of MHC (major histocompatibility complex) class 1 transmembrane proteins. They form a heterodimer with the β2 microglobulin protein (encoded by the B2M gene). The MHC class 1 molecules present short polypeptides, usually between 7-11 amino acids long, to the immune system for recognition as either "self" or "non-self". HLA-C, for instance, is present in all cells and exists as several haplotypes due to the diversity of HLA-C genes. C*08:02 represents one such haplotype. HLA class I present neoantigen-derived peptides to the cell surface, allowing them to be recognized by T cells, via TCR (T cell receptors). Cancer immunotherapy has been taking advantage of that mechanism, by engineering T cells to express TCRs able to recognize specific cancer immunogens. In 2016 the use of HLA-C*08:02-restricted TIL (tumor infiltrating lymphocytes) specifically targeting KRAS (Kirsten rat sarcoma virus) G12D mutation in lung cancer resulted in positive results. A similar approach was pursued in a patient with metastatic pancreatic cancer and resulted in regression of the disease. The study of HLA-C*08:02-restricted TIL expressing TCR against other neoantigens may prove beneficial in cancer therapy. K562 cells are HLA class I and II negative, making them an ideal cellular model to introduce and study specific haplotype responses. HLA mismatching between donor cells and the individual can lead to immune rejection, and one option is the knockout the endogenous HLA, allowing cells to be more widely universally used.

**Application**

- Knockout of HLA-A/B/C in cells of interest

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| | HLA-A/B/C gRNA | 10 µl | -20°C |
| | Cas9 enzyme (*Streptococcus pyogenes*) | 10 µl | -20°C |

**Storage Conditions**

Components are shipped in dry ice and stored at -20°C for long term storage. The components maintain their stability and performance after 5 freeze-thaw cycles.

**Materials Required but Not Supplied**

These materials are not supplied with the kit but may be required for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this kit and are highly recommended for the best results.





BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

| Name | Ordering Information |
|---|---|
| TCellM™ | BPS Bioscience #78753 |
| Normal Human Peripheral Blood Mononuclear Cells, Frozen | BPS Bioscience #79059 |
| Human Interleukin-2 Recombinant | BPS Bioscience #90184 |
| EasySep™ Human CD4+ T Cell Isolation Kit | Stemcell technologies #17952 |
| EasySep™ Human CD8+ T Cell Isolation Kit | Stemcell technologies #17953 |
| Human CD3/CD28/CD2 T cell Activator | Stemcell technologies #10970 |
| PE-labeled anti-human HLA-A,B,C Antibody | BioLegend #311405 |
| Neon NxT Electroporation System (or other electroporation system) | NEON1SK |

*Media Required for T cell Culture*

T Cell Medium: TCellM™ (BPS Bioscience #78753) supplemented with 10 ng/ml Human Interleukin-2 Recombinant (BPS Bioscience #90184).

**Assay Protocol**

- The following protocol was used to knock out the HLA-A/B/C in $CD4^+$ and $CD8^+$ primary T cells using the HLA-A/B/C Knockout Electroporation kit, and it is a general guideline only.
- The assay conditions (concentration of gRNAs and Cas9 enzyme, and time of assay) should be optimized according to the cell type, donor, and the assay requirements.

**Day 0:**

1. Isolate $CD4^+$ T cells and $CD8^+$ T cells from previously frozen human PBMC by negative selection, according to the manufacturer's instructions.

2. Mix $CD4^+$ T cells and $CD8^+$ T cells at a 1:1 ratio.

3. Culture cells in T Cell Medium at 1 x $10^6$ cells/ml, at 37°C in 5% $CO_2$ overnight.

**Day 1:**

1. Activate T cells with the Human CD3/CD28/CD2 T cell Activator reagent, following the vendor's recommendation and incubate at 37°C in 5% $CO_2$ for 24 - 48 hours.

**Day 3:**

1. Prepare the Cas9 RNP Complex in Resuspension Buffer R (provided with Neon NxT Electroporation System) as shown in the table below.

*Note: Always include extra volume to avoid pipetting errors and the creation of bubbles.*

| Component | Amount |
|---|---|
| HLA-A/B/C gRNA | 0.5 µl |
| Cas9 enzyme | 0.5 µl |
| Resuspension Buffer R | 4 µl |
| **Total** | **5 µl** |

2. Incubate the mixture at Room Temperature (RT) for 15 minutes.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and wash the cells with PBS.

4. Spin down the cells again, and resuspend the cell pellet in Resuspension Buffer R at a density of $4x10^7$ cells/ml.

5. For each reaction, add 5 µl of the cell suspension to 5 µl of the Cas9 RNP Complex Resuspension in Buffer R (from step 1).

6. Using the 10 µl Neon™ tip, pipet 10 µl of cells/Cas9 RNP complexes, and electroporate using an optimized protocol for primary T cells (1,600V, 10 ms, 3 pulses).

*Note: Avoid creating bubbles, which can affect the electroporation efficiency.*

7. After electroporation, immediately transfer the contents of the Neon tip into one well of the 6-well culture plate containing 2 ml of prewarmed T Cell Medium.

8. Incubate the cells at 37°C for 2-3 days.

**Day 5-6:**

1. Analyze the HLA-A/B/C knockout efficiency by flow cytometry, or other method of interest.

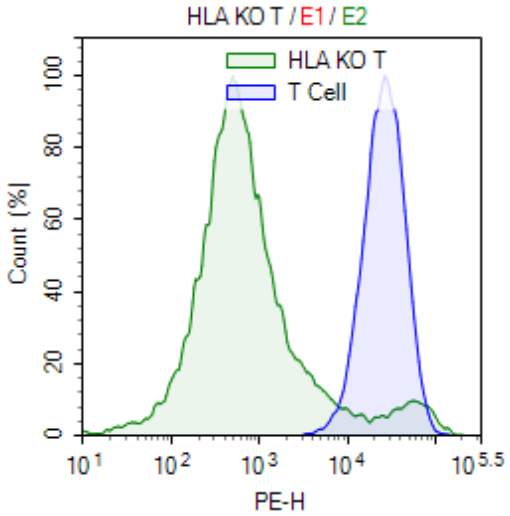


*Figure 1: HLA-A/B/C knockout in CD4⁺ and CD8⁺ T cells by flow cytometry.*
Approximately 250,000 CD4⁺ and CD8⁺ activated T cells were mixed with Cas9 RNP Complexes were electroporated using the Neon NxT Electroporation System. 72 hours after electroporation, the HLA-A/B/C expression was analyzed by flow cytometry using PE-labeled anti-human HLA-A, B, C Antibody (BioLegend #311405). Non-electroporated T cells were used as control (blue). T cells where TCR was knockout are shown in green. The y axis represents the % of cells, while the x axis represents the fluorophore intensity.

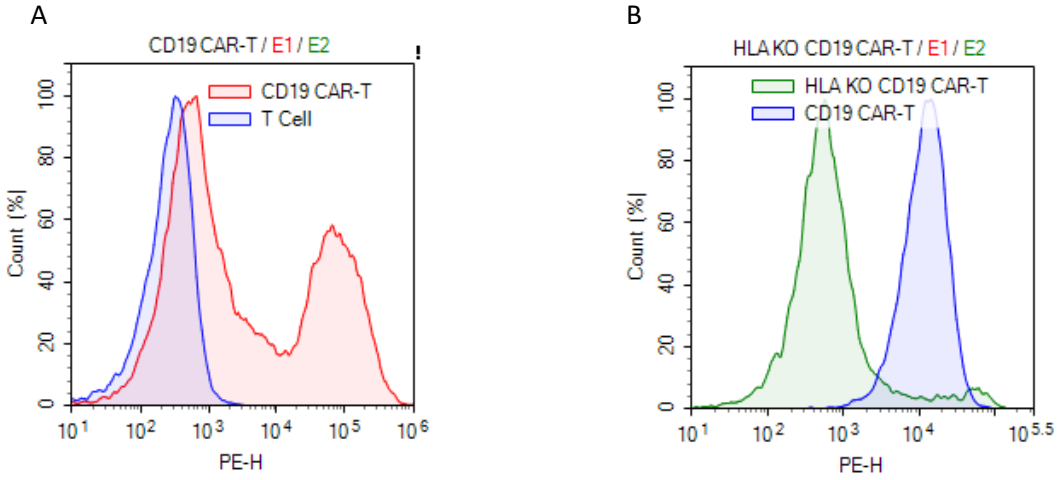


*Figure 2: HLA-A/B/C Knockout in CD19 CAR T cells.*
CD4⁺ and CD8⁺ activated T cells were transduced with Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector) (#78601). 72 hours after transduction, approximately 250,000 transduced cells were mixed with Cas9 RNP complexes and electroporated using the Neon NxT Electroporation System. 72 hours after electroporation, CD19 CAR expression was analyzed by flow cytometry with FITC-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (AcroBiosystems #FM3-FY45G0) (panel A), and the HLA-A/B/C expression was analyzed using a PE-labeled anti-human HLA-A, B, C Antibody (BioLegend #311405) (panel B).

| Gene Target | gRNA Sequence |
|---|---|
| HLA-A/B/C | CGGCTACTACAACCAGAGCG |

*Figure 3: gRNA sequence of the gRNA included in the HLA-A/B/C Knockout Electroporation Kit.*

**References**

Leidner R., *et al.*, 2022 *N Engl J Med* 386:2112-2119.
Tran E., *et al.*, 2016 *N Eng J Med* 375:2255-2262.

**Troubleshooting Guide**

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| TCR Knockout Electroporation Kit | 82394 | 1 kit |
| HLA-C*08:02 K562 Cell Line | 78974 | 2 vials |
| HLA-C*08:02 Lentivirus | 78930 | 500 µl x 2 |
| HLA-E Lentivirus | 78929 | 500 µl x 2 |

*Version 011425*