Jul 26, 2024 Version 3

T cell purification and activation V.3

DOI
dx.doi.org/10.17504/protocols.io.81wgbz431gpk/v3

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DOI: dx.doi.org/10.17504/protocols.io.81wgbz431gpk/v3

Protocol Citation: Moustafa Nouh Elemeery, Salix Boulet, louis-eric.trudeau Trudeau, Nathalie Labrecque 2024. T cell purification and activation. **protocols.io https://dx.doi.org/10.17504/protocols.io.81wgbz431gpk/v3**Version created by **Lilia Rodriguez**

Manuscript citation:
Elemeery, M. N., Tchung, A., Boulet, S., Mukherjee, S., Giguere, N., Daudelin, J. F., ... & Trudeau, L. E. (2024). Adoptive transfer of mitochondrial antigen-specific CD8+ T-cells in mice causes parkinsonism and compromises the dopamine system. *bioRxiv*, 2024-02.

Protocol status: Working
We use this protocol and it's working

Created: July 02, 2024

Last Modified: July 26, 2024

Protocol Integer ID: 104171

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP 000525

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Abstract

This protocol details the purification and activation of Mouse Naïve CD8+ T Cell from mouse spleens using a purification kit. The cells are then activated by culturing them in plates coated with anti-CD3 antibodies and adding soluble anti-CD28 antibodies. The activation process occurs over 72 hours, with IL-2 added after the first 24 hours to support T cell proliferation and survival. The isolation kit from STEMCELL is designed to isolate naïve CD62L+CD44-CD8+ T cells from single-cell suspensions of splenocytes by negative selection. Unwanted cells are targeted for removal with biotinylated antibodies that are directed against non-naïve CD8+ T cells (CD4, CD11b, CD11c, CD19, CD44, CD45R/B220, CD49b, TCRγ/δ, TER119) and streptavidin-coated magnetic particles. Labeled cells are separated using an EasySep™ magnet without the use of columns.

Materials

Reagents and solution:

	A	B	C
	Reagent or solution	Supplier	Catalogue #
	Anti-mouse CD3 (Clone: 145-2C11)	Bioxcell	BE0001
	Anti-mouse CD28 (clone: 37.51)	Leinco	C379-5.0 mg
	RPMI media	VWR (Corning)	CA45000-396
	Phosphate Buffer saline (PBS)	Gibco	14190144
	EasySep buffer	STEMCELL	20144
	EasySep™ Mouse CD8+ T Cell Isolation Kit	STEMCELL	19858
	ACK (Ammonium-Chloride-Potassium) Lysing Buffer	ThermoFisher	A1049201
	L-glutamine 200 mM	VWR	CA45000-676
	HEPES 1M	Fisher	MT25060CI
	Sodium Pyruvate	VWR	CA45000-710
	2-Mercaptoethanol	ThermoFisher	21985023
	Non-Essential Amino acids	VWR (Corning)	CA45000-700
	Fetal Bovin Serum	Gibco	12483020
	24 wells plate flat bottom suspension plates	Sarstedt	83.1836.500
	96 wells plate flat bottom suspension plates	Sarstedt	82.1581.001
	Cell strainers (70um)	Fisher	08-771-2

- InVivoMAb anti-mouse CD3 **BioXcell Catalog #**BE0001
- Anti-Mouse CD28 (Clone 37.51) **Leinco Catalog #**C379
- RPMI **VWR International Catalog #**45000-396
- Phosphate buffered saline (PBS) without Ca/Mg **Thermo Fisher Scientific Catalog #**14190144
- EasySep™ Buffer **STEMCELL Technologies Inc. Catalog #**20144
- EasySep™ Mouse Naïve CD8+ T Cell Isolation Kit **STEMCELL Technologies Inc. Catalog #**19858
- ACK Lysing Buffer **Thermo Fisher Scientific Catalog #**A1049201
- L(+)-Glutamine solution 200 mM **VWR International Catalog #**45000-676
- Sodium pyruvate solution 100 mM **VWR International Catalog #**45000-710
- 2-mercaptoethanol **Gibco - Thermo Fisher Catalog #**21985023
- Non essential amino acids **VWR International Catalog #**45000-700
- Fetal Bovine Serum, qualified, Canada **Thermo Fisher Catalog #**12483020
- Falcon™ Cell Strainers **Fisher Scientific Catalog #**08-771-2

RPMI complete (RPMIc):

	A	B
	RPMI	500 mL
	FBS 10%	50 mL (inactivated)
	L-Glutamine	5 mL
	Sodium pyruvate	5 mL
	Antibiotic (Pen-Strep)	5 mL
	Non-essential amino acids	5 mL
	HEPES	
	2-Mercaptoethanol	50 mmol/L (final)

Note

Note! Very important, 2-Mercaptoethanol is an essential growth factor for mouse T-lymphocytes.

Purification and activation

4d 0h 30m

1 Dilute CD3 antibody (145-2C11) (clone KT3 can also be used) to 1 undetermined in PBS.

2 Coat plates with anti-CD3 antibody.

3 Use 24 well (Sarstedt, cat# 83.1836.500) or 96 well (Sarstedt, cat# 82.1581.001) plates. If these plates are not used, there is a risk of partial stimulation due to low absorbance of the antibody on the plate.

3.1 Add 100 µL of antibody/well if 96 wells or 1 mL per well if 24 wells.

3.2 Incubate the plate for 24:00:00 at 4 °C or 01:00:00 at 37 °C . 1d

4 Remove the antibody (aspirate) and wash 2 times with PBS.

5 Add 100 µL of RPMIc if 96 wells or 1 mL if 24 wells and incubate the plate at 37 °C for 00:15:00 . See complete recipe for RPMIc in Materials section. 15m

6 Collect spleen from mice 6-8 weeks in complete RPMI media (RPMIc), sex matched with recipient mice.

Note: using spleens from 6-8 week old mice ensures that the isolated T cells are from young adult mice with a fully developed immune system, and the sex-matching helps prevent potential complications in downstream applications or experiments.

7 Purify CD8+ T cells using EasySep mouse naïve purification kit (STEMCELL, catalo # 19858) as follow:

7.1 Use a frosted microscope slide to homogenize spleens in PBS or Hanks' Balanced Salt Solution (HBSS) containing 2% fetal bovine serum (FBS).

7.2 Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer. Collect cells in a 15 mL tube.

7.3 Centrifuge at 1300 rpm, 00:05:00 and discard the supernatant. 5m

7.4 Red blood cells lysis is done by adding 5 mL /spleen of 0.83% ammonium chloride and incubate for 00:05:00 (or 00:02:00 for ACK lysing buffer) at Room temperature while continuously shaking tubes. 5m

7.5 Quench by filling up tube with RPMIc.

7.6 Centrifuge at 1300 rpm, 00:05:00 and discard supernatant. 5m

7.7 Resuspend in EasySep buffer at $1x10^8$ cells/ml (Easysep mouse naïve CD8+ T cell isolation kit (#19858A) and follow the protocol provided by STEMCELL.

8 Wash cells with RPMIc medium.

9 Resuspend cells in 5 mL of RPMIc, count cells and adjust the concentration to $2x10^6$ cells/mL

10 Add 100 µL of cells ($2x10^5$ cells) per well if 96 wells or 1 mL of cells ($2x10^6$ cells) per well if 24 wells).

11 Add purified anti-CD28 antibodies to reach a concentration of 5 undetermined .

12 Incubate the plate at 37 °C and 5% CO_2 for 24:00:00 . 1d

13 Add 20 Unit/ml of IL-2.

14 Incubate the plate at 37 °C and 5% CO_2 for another 48:00:00 . 2d

15

Note

***** Don't forget the non-stimulated controls.**

- Resuspend the cells well throughout the experiment (the cells quickly settle to the bottom of the tube)
- Check purity of CD8+ T cells after STEMCELL isolation by staining with anti-CD8 antibody followed by flow cytometry.
- Check activation after stimulation using anti-CD8 and anti-CD44 staining followed by flow cytometry.