This SOP replaces:	Date: 15.12.2018 Version: A
Reason for Change:	Protocol and related SOPs update
Related SOPs:	LTK-TRT-14-EN CO2 Euthanasia LTK-RES-1-EN EAE Scoring LTK-TRT-10-EN ip injection LTK-RES-26-EN EAE induction
Indication of Use:	Performing an adoptive transfer EAE experiment
Aim of SOP:	Obtain encephalitogenic T lymphocytes and transfer them into a host for the induction of EAE (experimental autoimmune encephalomyelitis).
Distribution:	1. Original: Server 2. Copy: Work place
Attachments:	

Generated at: 22.02.2022	Checked and approved at: 23.02.2022
by: Vasileia Kalaitzaki	by: Thorsten Buch

3

Responsible Persons: person registered on license

Principle of Method: encephalitogenic T cells are generated from ex vivo myelin-specific T cells.

Material to be used: Splenocytes from donor mice (1 donor for 4 hosts).

Min/Max amount: e.g., Inject i.p. 100 to 150 million cells/mL in a total of 200 to 300 µL

Materials and reagents:

14-16 µg rmIL-12 (recombinant murine IL-12 (Cat#210-12 Peprotec)
7-8 mg Blocking anti-mouse IFNγ antibody (XMG1.2)
6 70 µm cell strainers (BD/Falcon #352350)
7 to 8 TC flasks (Corning #430825)
RPMI 1640 (Sigma R8758-500ML RPMI-1640)
L–Glutamine–Penicillin–Streptomycin solution (Sigma #G6784)
MEM Non-Essential Amino Acids Solution (Life Technologies #11140-050)
MEM Sodium Pyruvate Solution (Life Technologies #11360-070)
Red Blood Cell Lysing Buffer (Sigma #R7757)
Sterile phosphate buffered saline (PBS)
70% isopropyl alcohol in spray bottle
50 mL sterile polypropylene tubes
Pipettes - 5 mL, 10 mL, 25 mL
Media bottles
Petri dishes (Sarstedt cat#82.1473)

Method Description:

Induce EAE in donor mice as per SOP-LTK-RES-26-EN EAE induction.

At day 11, euthanize the mice as per LTK-TRT-14-EN Euthanasia, collect the spleens and isolate the cells. Perform all cell preparation steps under sterile conditions in a biosafety cabinet. Keep cells cold (0 to 4 °C). Use cold media and keep cells on ice.

1. Place spleen in a strainer inside a petri dish. Squash spleens from 4 to 5 mice in the Petri dish by pressing several times with the hard end of a 10 mL syringe plunger. Place a fresh 70 µm cell strainer in a 50 mL tube. Collect all media and squashed tissue from the Petri dish into the cell strainer.

2. Spin down cells in 50 mL tubes for 10 minutes at approximately 300 g. Discard supernatant and resuspend the cell pellet in 2.5 to 3 mL per mouse of cold red blood cell lysing buffer and keep cold for 4 to 5 minutes while red blood cells lyse.

3. Immediately after cell lysis is complete add 35 mL of cold wash media to the cell suspension in each tube. Spin down cells again for 10 minutes at approximately 300 g, then discard supernatant.

4. Resuspend the cells in ~5 mL tissue culture media (RPMI 1640 supplemented with L–Glutamine–Penicillin–Streptomycin) per mouse. Again, filter suspension through a fresh 70 µm cell strainer to remove clumps of cells. Count cells with on Nexcelom chambers with automated cellometer using Trypan Blue solution. Expected cell number is approximately 200 million cells from each donor mouse.

5. Transfer cells from all tubes into a single media bottle and dilute with tissue culture media to 3–3.5 million cells/mL.

1. Add $MOG_{35–55}$ peptide to the cell suspension to reach 20 µg peptide/mL..

2. Add recombinant mouse IL-12 to the cell suspension to reach a concentration of 20 ng/mL.

3. Add anti-mouse IFNγ antibodies to the cell suspension to reach 10 µg/mL.

4. Plate the cell suspension (3–3.5 million cells/mL) at 100 mL/TC flask (T150) and culture for 70-72 hours, 37 °C, 5% CO2, humidified.

Transfer cells to recipient mice

1. Collect cells from TC flasks into 50 mL tubes. Spin down cells, 10 minutes at approximately 300 g.

2. Resuspend cells in approximately 2 to 3 mL PBS per flask.

3. Count cells with on Nexcelom chambers with automated cellometer using Trypan Blue solution. Expected cell number is approximately 100-120 million cells from each donor mouse.

4. Adjust cell concentration with PBS. For i.p. injection, adjust to 100 to 150 million cells/mL.

5. Inject cell suspension into recipient mice i.p. at 0.2 to 0.3 mL/mouse (SOP-LTK-TRT-10-EN ip injection).

6. Begin scoring mice 5 days after the cell transfer as per SOP LTK-RES-1-EN EAE Scoring

Controls:
Positive control - C57BL6 mice should develop EAE
Negative control - Mice that received a i.p. injection of PBS only

Criteria for approving outcome:
C57BL6 mice should develop EAE within 9 days.

Analysis:
Daily scoring as per SOP-LTK-RES-1-EN EAE Scoring

Documentation:
Server

Method validation:
Server: EMM AT1-AT4, EMC AT1-AT2

Literature:
https://hookelabs.com/protocols/adoptiveTransferEAE_C57BL6.html