This SOP replaces:	Date: A
Reason for Change:	Response to veterinary office
Related SOPs:	SOP LTK-TRT-7 iv injection SOP LTK-TRT-14 CO_2 euthanasia
Indication of Use:	Determination of tumor responses triggered by adoptively transferred cells
Aim of SOP:	This protocol describes how to perform mouse treatment by adoptive transfer of cells

Distribution:

1. Server
2. Animal facility
3. Group vom Berg

Attachments:

Generated at: 07.11.2022	Checked and approved at: 08.11.2022
by: Michal Beffinger	by: Johannes vom Berg

Responsible Persons:
1) The researcher mentioned on the respective scoring sheet.
2) Any person with Module 1 and registered on animal permit.

Method: Preparation of cells for adoptive transfer

Principle of Method: Cells for adoptive transfer are purified from organs of donor mice and prepared for injection into tumor-bearing animals.

Material to be used:
PBS
50 mL sterile polypropylene tubes
Pipettes - 5 ml, 10 ml, 25 ml
Syringe 10 ml
70 µm cell strainer
Magnetic cell separation kit

Storage of Material:
Follow the individual data sheets for all chemicals used.

Machine:
Centrifuge
Magnets for magnetic cell separation

Safety:
1. General rules for working with sharp tools (scalpels, syringes, scissors) have to be followed.
2. Follow the rules of the animal house.
3. Follow the rules of working with toxic chemicals.

Method Description:

Perform all cell preparation steps aseptically in a biosafety cabinet. Keep cells cold (4°C). Use cold PBS and keep cells on ice when practical.

1. Euthanize donor mouse and collect the spleen (see **SOP LTK-TRT-14 CO2 euthanasia**)
2. Place spleen in a strainer inside a petri dish. Squash spleen 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 PBS and squashed tissue from the Petri dish into the cell strainer.
3. Spin down cells in 50 ml tubes for 5 minutes at 350 g. Discard supernatants.
4. Purify cells using magnetic cell separation. E.g., for purification of OT-I cells use $CD8^+$ T cell purification kit, for purification of OT-II cells use $CD4^+$ T cell purification kit.
5. Count the cells and resuspend in PBS to reach up 10^7 cells per mouse and volume up to 5 µl/g of mouse body weight.
6. Inject cells i.v. according to **SOP LTK-TRT-7 iv injection**

Documentation:
Server, appropriate project folder.

Problem management:
Report any adverse event to your supervisor