University of Zurich[UZH] Institute of Laboratory Animal Sciences	**Standard Operating Procedure** **SOP**	**Page 1 of 5**
Date: 26.01.2022	**Bone Marrow Chimerism** **BMT**	**LTK-RES-2-C-EN** **Version: C**

This SOP replaces:	Date: 08.09.20 Version: B
Reason for Change:	Provide more details
Related SOPs:	SOP-LTK-TRT-7-EN i.v. injection SOP-LTK-RES-1-EN EAE Scoring SOP-LTK-TRT-14-EN Euthanasia
Indication of Use:	Replacement of the hematopoetic system of a mouse with that of of another mouse. Either of the two mice may have different gene deficiencies/modifications (or none).
Aim of SOP:	This procedure describes how to repopulate a mouse with another hematopoetic system

Distribution:

1. Original: Thorsten Buch
2. Copy: Animal facility
3. Intranet

Attachments:

Generated at: 24.01.2022	Checked and approved at: 26.01.2022
by: Antonios Katsoulas	by: Thorsten Buch

Responsible Persons: Any person with Module 1 and registered on a particular animal permit

Method: irradiation, i.v. injection, antibiotic treatment

Principle of Method: The original hematopoetic system is removed by irradiation. Hematopoetic stem cells or bone marrow cells of another individual are injected intravenously. Within 9 weeks the animals are repopulated with new bone marrow

Units and Formulas: Rad

Material to be used:

1. For many experiments it is useful to use RAG-deficient mice. Upon reconstitution all T/B cells are derived from the donor. Radioresistant myeloid tissue-resident cells may still be from the recipient.
2. Often the material is from a donor mouse that is deficient in a certain gene.
3. By use of surface markers such as CD45.1 and CD45.2 or CD90.1 and CD90.2 it is possible to identify the generated mixed bone marrow chimeras.

Min/Max amount: The minimum and maximum donor cells for efficient reconstitution must be determined experimentally. The bare minimum is, however, $1x10^6$ cells per host mouse.

Material acquisition: Donor cells for reconstitution are obtained as described below.

Calibration: Ensure that the irradiation device is properly calibrated (done by the facility). Introduction is mandatory

Storage of Material: Material should be used as fast as possible after acquisition. Kept on ice while being obtained but injected at room temperature.

Machine:
Laminar flow
Centrifuge
Counting chamber + Inverse microscope or cytometer such as Cellometer 1000
Laminar flow/changing station
Irradiation facility

Material:
dissection scissors
dissection forceps
Counting chamber

Reagents:
PBS
Borgal

Safety:
Follow the rules for the animal house
Follow the rules for the irradiation unit, get the required introduction

Method Description:

Preparation of recipient mice

1. Irradiate mice belonging to the same cage together. Cover the floor of the cage with tissue paper.
2. Under the laminar flow hood, transfer the mice to the radiation cage (kept within the animal facility in Schlieren). Wash the cage with ethanol before using it for irradiating mice belonging to a different cage. Replace the tissue paper.
3. Irradiate twice with a 24h interval. Per irradiation, give 550 rads (total 1100 rads).

NOTE: follow the instruction of the irradiation facility/machine.
For the “Rad score 2000” machine at room WAF D153 (Schlieren), use the program “A” in the irradiation machine: 225kV, 17.8mA. Set the irradiation time for 2 min 57s.

Preparation of BM

As soon as the second radiation ends, start preparing the donor cells. Aim for the shortest possible interval between the second irradiation and the transfer of the donor cells.

1. Euthanize BM-donors (LTK-TRT-14-EN SOP CO2 Euthanasia)
2. Dissect skin and muscle from femur and tibia
3. Cut femur from tibia and flush each bone with sterile PBS
4. Force cell suspension through a 70 µ strainer prior to centrifugation
5. Count cells and resuspend @ 25 x 10^6 cells/mL. Account for approximately 100 µL per host mouse (see below).

Transplantation of BM

1. Inject a maximum of 100 µL per 20 g of body weight.

NOTE: See LTK-TRT-7-EN SOP i.v. injection

Antibiotic Treatment

1. Start antibiotic treatment after the second irradiation.
2. Add 250 µL ml Borgal into 1 bottle drinking water (250 mL)
3. Keep the treatment for 21 days (minimum) but change water/antibiotic 1x a week.

Recovery and Observation

1. Follow LTK-TRT-8-EN SOP Scoring post-application.

NOTE: Take mice out of experiment when they reach minimal critical weight

2. In order to achieve >95% engraftment, the mice are led to recover for 8 weeks prior to experimentation

Controls:
Transfer of Wildtype cells into Wildtype hosts (usually it is the positive control)
Transfer of mutant cells into mutant hosts (usually it is the negative control)

Factors influencing outcome:
Age of the donors and host. Younger mice are preferred, but should be pre-determined
Failing to inject the full volume of cells per mouse (refer to LTK-TRT-7-EN SOP i.v. injection).

Criteria for approving outcome:
Full reconstitution, as accessed by FACSs of a blood sample, one week prior to downstream experimental processes.

Analysis:
Flow cytometry

Documentation:
openBIS, Score sheet SOP-LTK-RES-1-EN EAE Scoring

Problem management:
Report any adverse event to supervisor or vet

Literature:
J. Exp. Med. 2001, 93/8, 967-974.
J Mol Med (Berl). 2019 Jul;97(7):889-89