 University of Zurich <small>UZH</small> Institute of Laboratory Animal Sciences	Standard Operating Procedure SOP	Page 2 of 5
Date: 07.01.2015	Bone Marrow Chimerism BMT	LTK-TRT-9-A-EN Version: A

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

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

Principle of Method: The original hematopoietic system is removed by irradiation. Hematopoietic stem cells or bone marrow cells of another individual are injected intravenously. Within 9 weeks the animals are repopulated with a 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. By use of surface markers such as CD45.1 and CD45.2 or CD90.1 and CD90.2 it is possible to generate mixed bone marrow chimeras.


Min/Max amount:

Material acquisition:

Calibration: Ensure that the irradiation device is properly calibrated. Obtain an introduction.

Storage of Material:

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Machine:
Laminar flow
Table top centrifuge
Counting chamber
Inverse microscope
Laminar flow/changing station
Irradiation facility

Material:
dissection scissors
dissection foresceps
Counting chamber

Reagents:
HBSS
Borgal

Safety:
Follow the rules for the animal house
Follow the rules for the irradiation unit

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Method Description:

Preparation of recipient mice

1. Irradiate mice under SPF-conditions in filter-cage with 550 rads. Repeat irradiation 24 hours later. (total 1100 rads)

Preparation of BM

1. Euthanize BM-donors (SOP CO2 Euthanasia LTK-TRT-14-A-EN)
2. Dissect skin and muscle from femur and tibia
3. Cut femur from tibia and flush each bone with sterile HBSS
4. Force cell suspension through a 70 µ strainer prior to centrifugation
5. Count cells and resuspend @ 40-60x10⁶ cells/ml

Transplantation of BM

- See SOP i.v. injection LTK-TRT-7-A-EN
- Inject a minimum of 10⁶ BM cells/ recipient

Antibiotic Treatment

- 1 ml Borgal (24%) into 1 bottle drinking water (250 ml) over 21 days
- change bottle every 3 days

Recovery and Observation

- Follow SOP "Scoring post-application" LTK-TRT-8-A-EN
- In order to achieve >95% engraftment, the mice are led to recover for 6-8 weeks prior to experimentation

Controls:

Wildtype → Wildtype
Mutant → Mutant


Factors influencing outcome:

-

Criteria for approving outcome:

full reconstitution

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Analysis:
Flow cytometry

Documentation:
Lab book, Score sheet SOP scoring post-application

Problem management:
Report any adverse event to your supervisor or vet

Sample storage:
-

Method validation:
-

Literatur:
J. Exp. Med. 2001, 93/8, 967-974

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