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Date: 18.02.2021	tail bleeding	LTK-TRT-19-C-EN Version: C

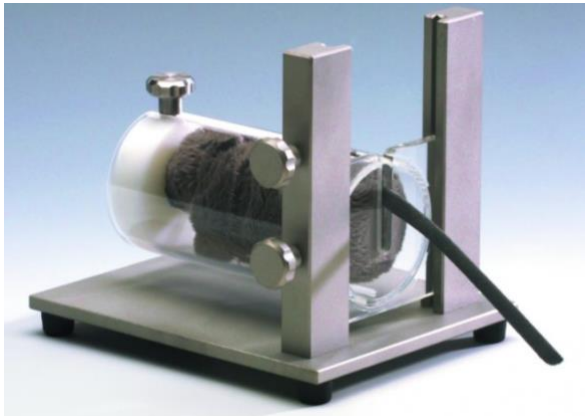
Responsible Persons: Researcher with Modul 1 after registration on animal license

Method: bleeding

Min/Max amount:
 The maximum volume of blood collected is 1% of body weight (BW, 200 µl / 20 g mouse)
 Examples: With 1% of BW bled, next bleeding after 2 weeks, With 0.5% of BW bled, next bleeding after 1 week, with less than 0.25 % (50 µl / 20 g mouse) after 48h. For immunological assays such as ELISA and flow cytometry 50 µl is sufficient.

Material:


1. scalpel, straight edge razor
1. heparinized whole blood collection tubes (orange cap, BD) or serum separator tubes (yellow cap, BD)
2. restrainer (1)



3. water in beaker at 42°C

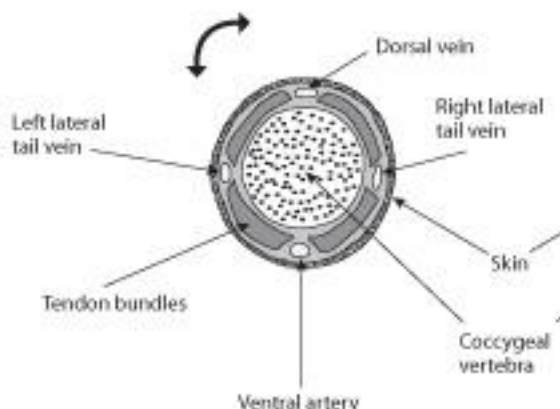
Safety:

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

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Method Description:

2. Warm the tail of the mouse in 42°C warm water.
3. Restrain the mouse in the physical restrainer device or anaesthetize the mouse (if required by permit).
4. Using a scalpel, straight edge razor, or sharp scissors, quickly nick the lateral tail vein (see transverse section below, 2) at the distal part of the tail close to the tip.



5. Collect blood in a capillary tube as drops appear. Do not attempt to increase blood flow by rubbing the tail from the base to the tip, as this will result in leukocytosis (increased white blood cell count)
6. Collect up to 1% of body weight (200 µl / 20 g mouse) of blood into a collection tube (see materials)
7. take a sterile swab and apply pressure on the wound to stop the bleeding
8. Gently release mouse from the restrainer and place it back into the home cage

Documentation:


Lab Journal, iRATS - put animal into experiment and project, severity 1

Problem management:

In case of serious adverse events, contact supervisor, lab head or vet.

Sample storage:

- Do not freeze samples before you have separated serum. Freeze serum at -80°C for later analysis by ELISA or WB
- Keep blood samples for FACS at 4°C until analysis.

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Literatur:

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2. The Laboratory Mouse, Chapter 32: routes of administration, Shinya Shimizu, figure 32.12 a, Copyright 2004, Elsevier ISBN 0-1233-6425-6
3. Dürschlag M, Würbel H, Stauffacher M, Von Holst D. Repeated blood collection in the laboratory mouse by tail incision--modification of an old technique. *Physiol Behav.* 1996 Dec;60(6):1565-8. PubMed PMID: 8946506.
4. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van de Vorstenbosch C; European Federation of Pharmaceutical Industries Association and European Centre for the Validation of Alternative Methods. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol.* 2001 Jan-Feb;21(1):15-23.
5. fachinformation 3-02-blutentnahme-zu-versuchszwecken_DE.pdf, downloaded from BLV 22.01.2021