Vascular Perfusion of Mice

Version: 1
Replaced by version: N/A
Edited by: Peter Mundel

Summary: This protocol describes the procedure for perfusion of mice prior to organ or tissue harvesting .

Reagents and Materials:

Reagent/Material	Quantity Required	Vendor	Stock Number
Perfusion Apparatus	1		
Perfusion Bottles	2		
Small diameter tubing (< 1cm)			
butterfly 23 gauge ¾	1		
Ketamine	3 ml		
Xylazine	1 ml		
Plastic Tray	1		
Phosphate Buffered Saline, pH 7.4			
PFA			
Sucrose			
70% alcohol			
Scissors			
Cotton swabs			

Protocol:

Preparation (see figure below for setup)

- Fix tubing (small diameter tubes, below 1 cm) to the bottles: 1 bottle for PFA, 1 bottle for sucrose
- Fill perfusion solutions in the bottles: solutions must be freshly prepared and filtered (using 0.22 µm filter)

- Adjust perfusion pressure to 150-160 mm Hg
- Fix needle at the end of tubing after valve: butterfly 23 gauge 3/4
- **Remove any air from all tubing!!**
- Anesthetic: ketamine-xylazine mix (150/10 mg/kg=0.1 ml/20 g BW)
- Mix 3 ml ketamine 100mg/ml + 1 ml xylazine 20mg/ml + 6 ml sterile saline
- Fix the anesthetized animal in a plastic tray
- Solution #1: 4 % PFA in PBS, ph 7.4
- Solution #2: 18% Sucrose in PBS, ph 7.4

Perfusion:

- Swab mouse with 70% alcohol to wet the fur, cut open abdominal skin by a longitudinal incision
- Remove skin and gut to expose abdomal aorta and Vena cava
- Clean the aort abdominalis and vena cava from connective tissue and fat with cotton swabs
- Clamp aorta abdominalis and vena cava **in the area of the iliac arteries**
- Insert the **butterfly 23 gauge 3/4** needle in the Aorta below the renal arteries
- Immediately cut open the Vena cava with small and sharp scissors: this should allow a rapid drain of the perfusion solutions!
- Watch the needle carefully during the entire perfusion!!!
- Start perfusion for 3 minutes with PFA, pH 7.4 at 37^0C
- Switch valve to Solution #2:
- Without any interruption of pressure/flow perfuse for 5 minutes with sucrose, 7.4 at 37^0C
- Stop perfusion, remove butterfly
- Cut out kidneys at renal hilum for further processing
- Before starting to perfuse the next animal rinse the tubing and needle extensively

Schematic of custom-made perfusion system