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Vascular perfusion of mice V.2

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We use this protocol and it's working

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Abstract

Summary:

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

Diabetic Complications:

Cardiovascular

Retinopathy

Neuropathy

Nephropathy

Uropathy

Materials

Reagents and Materials	Quantity Required
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	

1 **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

2 **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 37ºC

♦ Switch valve to Solution #2:

♦ Without any interruption of pressure/flow perfuse for 5 minutes with sucrose, 7.4 at 37ºC

♦ 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

3 **Schematic of custom-made perfusion system**