Vascular perfusion of mice V.2

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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

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Protocol status: Working
We use this protocol and it's working

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Keywords: Vascular perfusion, cardiovascular, retinopathy, neuropathy, nephropathy, uropathy

MATERIALS

<table>
<thead>
<tr>
<th>Reagents and Materials</th>
<th>Quantity Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion Apparatus</td>
<td>1</td>
</tr>
<tr>
<td>Perfusion Bottles</td>
<td>2</td>
</tr>
<tr>
<td>Small diameter tubing (&lt; 1cm)</td>
<td></td>
</tr>
<tr>
<td>butterfly 23 gauge ¾</td>
<td>1</td>
</tr>
<tr>
<td>Ketamine</td>
<td>3 ml</td>
</tr>
<tr>
<td>Xylazine</td>
<td>1 ml</td>
</tr>
<tr>
<td>Plastic Tray</td>
<td>1</td>
</tr>
<tr>
<td>Phosphate Buffered Saline, pH 7.4</td>
<td></td>
</tr>
<tr>
<td>PFA</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
</tr>
<tr>
<td>70% alcohol</td>
<td></td>
</tr>
<tr>
<td>Scissors</td>
<td></td>
</tr>
<tr>
<td>Cotton swabs</td>
<td></td>
</tr>
</tbody>
</table>

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 abdominal 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