PROTOCOL FOR: CARDIAC IMAGE-GUIDED NEEDLE INJECTION ON ADULT MICE USING THE VEVO 770®

The following recommendation describes the key methods and materials required to perform high-resolution image-guided injection of mice using the VisualSonics’ Vevo™ 770 high-resolution in vivo imaging system. This recommendation uses Isoflurane as an anesthesia. Several publications include the use of injectable anesthesia including pentobarbital and could be used for the image-guided needle injection procedure.

Abstract: The Vevo 770 high-resolution imaging system and integrated Vevo Rail System II and Image-Guided Needle Injection Apparatus provides the required tools to perform injection of cells into the left ventricle of an adult mouse under image guidance. Please note that this technique can also be used for the guidance of injection and extraction procedures in mice embryos and pups as well as in other small animal models such as the rat.

1.0 Instrument Layout:

Vevo Compact Anesthesia System Set-Up including compressor, induction chamber, hosing, dissipation canisters and nose cone integrated with the Vevo Mouse Handling Platform.
**Vevo Integrated Rail System II with Injection System** including completed Vevo Rail System II, mounted 700-Series RMV scanhead, Mouse Handling Platform on Ball Joint and Quick-Lift mount, Physiological Controller unit and Injection unit and controller.

**Vevo 770 High-Resolution Imaging System** including rolling cart, monitor and two mounted 700-Series RMV scanheads.
2.0 **Materials**

The following materials are required for the image-guided needle procedure:

- Depilatory Cream
- 10cc Syringe
- 30 gauge needle
- Gauze

3.0 **Methods:**

*a) Imaging Preparation*

1. Start up the Vevo 770 Imaging System and load parameter file (if applicable)
2. Turn the temperature on the Mouse Handling Table to 40 degrees Celsius.

*b) Inducing Anesthesia*

3. Place mouse in induction chamber. Turn oxygen regulator to 1L/min and set anesthesia to 2.5%.
4. After 1-2 minutes in the induction chamber, perform the paw pinch test. Once the mouse has passed the test, move it to the Mouse Handling Table, redirect the anesthesia to the nose cone using the manifold and insert the mouse’s nose into the cone.

*c) Animal Preparation*

5. Apply corneal lubricant to the mouse’s eyes to protect them from the light during the procedure.
6. Tape paws to the ECG metal strips on the Mouse Handling Platform.
7. Apply lubricant to the rectal temperature probe and carefully insert it. This will be used to monitor the mouse’s temperature.
8. Remove hair from the animal’s chest.
9. Wipe the animal’s chest down using a damp piece of gauze.

*d) Detailed Injection Procedure*

10. With nothing on the Vevo screen (i.e. the animal is out of plane), slowly bring the needle into view.
11. With the needle at the focus, mark with the tip of the needle with the needle injection overlay.
12. Retract the needle using the micromanipulator.
13. Bring the left ventricle into view via a parasternal long axis and line up the target (in this case the apex) with the caliper indicating the needlepoint.
14. Slowly bring the needle into plane.
15. When the needle is close to the edge of the animal’s skin, turn the micromanipulator in a quick motion in order to puncture through the skin and the heart’s wall. The procedure will be visualized on screen.
16. Slowly retract the needle.

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