

SOP No: AHP 73(a)

SUBJECT: Transcardial perfusion in the mouse

POLICY: This procedure may only be performed by operators skilled in the technique.

PRECAUTIONS: Surgical gloves, eye protection, long-sleeved gown, closed in shoes.
This technique must be performed in a fume cabinet.

EQUIPMENT: small and large forceps
perfusion pump with 29g needle attached to the end spatula
falcon tubes
tray with cork board and green tray underneath
4% PFA (150ml per animal)
0.1M PBS (20ml per animal)
bone cutters
lethabarb
cryostat cutting compound (OTC Tissue Tek)
-80°C freezer

PROCEDURE:

1. Mice are given a sub-lethal dose of anaesthetic or lethabarb i.p. using a 26g needle.
2. Adequate anesthesia is determined by the eye blink method and the foot-pad squeeze test. The heart must still be beating.
3. Make an incision through the sternum and lift the rib cage to expose the heart (cut through with the blunt side of the scissors). Release the heart from the surrounding tissue. The rib cage can then be either cut off or held back with hemostats.
4. A blunt 29 gauge needle (made by removing the end 1 cm of the needle with hemostats and then filed down) connected to a peristaltic pump (Cole Parmer Instruments, Illinois, USA) primed with 0.1M Phosphate Buffered Saline (PBS) is pierced through the left lateral ventricle diagonally into the ascending aorta and is clamped down at the LV to hold the needle in place. An incision can be made in the right atrium to allow the fluid to flow through.
5. 15-20mL of 0.1M PBS (Phosphate buffered saline) is pumped through at a rate of 52mL/min (level 3 on the pump) to clear the blood from the animal followed by 80-100mL of 4% Paraformaldehyde (PFA).

- 6. Upon completion of the perfusion the animal is decapitated and the brain removed.**

RECOMMENDATIONS:

DATE ISSUED: **04.12.2008**

REVISED:

REFERENCES

1. DeMarch, Z. Giampa, C. Patassini, S. Bernardi, G and Fusco, F. R. (2008) *Neurobiol Dis* 30, 375.
2. Jackson-Lewis, V and Przedborski, S. (2007) *Nat Protoc* 2, 141.
3. Nakase, T. Fushiki, S. and Naus, C. C. (2003) *Stroke* 34, 1987.