

Research Animal Standard Operating Procedures (SOP) must meet the following criteria:

1. Describe procedures or activities involving research animal(s) common to more than one research project.
2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

Name of Procedure	Intraperitoneal anaesthesia – ketamine/xylazine	
Species	Mouse	
ACEC	Reference	SOP#7-Feb22-Intraperitoneal anaesthesia with ketamine/xylazine – Mouse
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	Version	1.3
	Date approved	25 February 2022
	Date for review	24 February 2025
	Procedure classification 1. Observation involving minor interference 2. Animal unconscious without recovery 3. Minor conscious intervention 4. Minor surgery with recovery 5. Major surgery with recovery 6. Minor physiological challenge 7. Major physiological challenge	3
Ethical considerations	1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes. 2. The procedure must be performed according to current best practice to support the wellbeing of the animal. 3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent.	

Details

Description of procedure

1. Materials:

- 1.1 1mL syringes
- 1.2 27 gauge needles

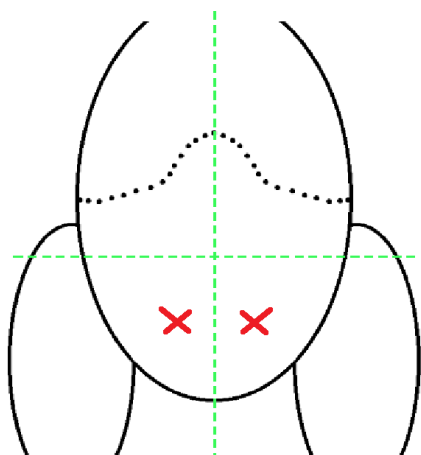
2. Reagents:

- 2.1 Xylazine 20mg/mL
- 2.2 Ketamine 100mg/mL
- 2.3 Sterile PBS
- 2.4 Ketamine/Xylazine WORKING SOLUTION (prepare fresh each time)

To prepare 2 mls of ketamine/xylazine mixture: Mix 180 ul Ketamine (100mg/ml) + 100 ul Xylazine (20 mg/ml) + 1720 ul sterile PBS. Label this as “Ketamine/ Xylazine Working Solution” and store at 4°C until use. Discard at end of day.

3. Method:

- 3.1 Remove an animal from its cage, and place it on the lid of the cage. Hold the tail of the animal then grasp the mouse firmly by the neck with your thumb and pointer finger. Lift the animal from the cage lid and gently turn on its back so you are holding the tail firmly with your thumb and supporting the mouse in the palm of your hand.
- 3.2 Locate approximately 2mm lateral to the umbilicus for mice, in the lower half of the abdomen. This is the site for intraperitoneal (IP) injection.



Picture demonstrating location for ip injections.
The head is at the top of the picture.
The black dotted line represents the rib cage.
The green dotted lines divide the abdomen into top and bottom; left and right quadrants.
The red 'X' marks indicate possible injection sites

- 3.3 With the bevelled edge of the needle facing upwards, insert the needle shallowly to ensure that it does not enter an abdominal organ. This is best achieved by placing the needle almost parallel to the abdominal wall when injecting.

NOTE: To avoid inadvertent intravenous injection, the syringe plunger should be drawn back after introducing the needle into the tissue. If the needle has penetrated a vessel, blood will be seen in the hub of the needle.

- 3.4 Give the Ketamine/Xylazine Working Solution at a rate of 0.2mL/ 20g of body weight using a 1mL syringe and a 27 gauge needle.
- 3.5 For lighter anaesthesia use 0.1mL/ 20g of body weight.
- 3.6 Once procedures have been completed and mice are yet to recover from anaesthesia, ensure mice are kept warm by use of heat lamp or heater until recovery.

NOTE: The degree and length of anaesthesia obtained in any animal depends on many factors, including individual and strain variations. The best dose for a given procedure depends on many factors. Some of these factors are:

- 1) the length of time the rodent must be anaesthetised
- 2) the level of pain that will be inflicted on the rodent
- 3) the sensitivity of that particular rodent to the anaesthetic agent.

The dose for any particular animal or group of animals must be titrated to give effective anaesthesia for the necessary length of time.

Drug details

Drug name (generic name, not trade name)	Dose rate (mg/kg body weight)	Route	Timing of administration, and frequency (<i>e.g. 30 minutes pre-operative, to induce anaesthesia, during procedure, at specific intervals during the procedure</i>)
Mixture of Ketamine and Xylazine.	10mg/kg xylazine + 80 -100 mg/kg ketamine	IP injection	Initial injection given just prior to procedure. Top-up doses given each 40-60 minutes as required.

Monitoring

Clinical or physiological criteria that will be used to monitor the depth of anaesthesia and general well-being of the animal during the anaesthesia.

- Respiratory frequency will be monitored to ensure slow constant breathing.
- The adequacy of the depth of anaesthesia will be checked intermittently using lack or reflexes such as the withdrawal reflex (flexion of the leg following a firm pinch of the paw or interdigital skin) or the palpebral reflex (in response to stroking the eyelids).

Monitoring of animals to ensure satisfactory recovery from anaesthesia.

Mice will be monitored until regular levels of activity and awareness are achieved.

ACEC Chair

