

 THE UNIVERSITY OF QUEENSLAND AUSTRALIA CREATE CHANGE	UQ Animal Ethics Committee - Standard Operating Procedure LAB_096 Injections – Mammary Fat Pad in Rodents Institutional author: UQ Biological Resources AEC Reviewed & Approved: 07/05/2026 SOP Expiry: 07/05/2029	Version #3.2 Page 1 of 7
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LAB_096 Injections - Mammary Fat Pad in Rodents (Expiry: May 2029)

I. OBJECTIVE

To describe the standard Mammary Fat Pad injection procedure in rodents used across UQ research projects, also reflecting the procedure used to train workers across UQ by UQBR.

NB: The use of (*) indicates this statement is dependent on the facility procedures

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II. DEFINITIONS

Competent - “the consistent application of knowledge and skill to the standard of performance required regarding the care and use of animals. It embodies the ability to transfer and apply knowledge and skill to new situations and environments.”¹

III. COMMENTS / RECOMMENDATIONS

- **Aseptic technique** should be used in making up solutions, dilution of substances, drawing up the substance and injecting the animal. It should also be used to prepare the skin if there is a risk of infection. For example, when injecting tumour cells, biologicals or jells - Clip the hair, clean with antiseptic such as chlorhexidine or betadine, wipe with 70% ethanol.
- **Clean technique** is typically adequate when preparing the skin i.e. make sure skin is clean and dry - wipe skin with 70% ethanol or similar if this is appropriate for the substance being injected.
- Mammary Fat Pad injections must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- The closed or open method of performing this procedure should be chosen prior to commencing.
- Generally mature females are used in this technique to increase accuracy of injection due to improved ability to identify the fat pad.
- After drawing up a substance, a new needle should be used to inject the animal. This is to ensure a sharp needle and minimise contamination.

Table 1. Recommended values for Mammary Fat Pad Injection in Rodents		
Needle Gauge	30-25G	A 25G needle is ideal for this work, it will allow the substance to be injected at a steady pace.
Needle Length	12mm	
Needle Depth at Injection	2.5mm	
Max Injection Volume	Project based upon approval, 30-50ul is a general range	
Attempt allowance	A maximum of two attempts unless otherwise approved by the Animal Ethics Committee (AEC)	
Procedure number and frequency	This is highly dependent on the project e.g if developing tumours, the impact of on mobility, body systems and organs. As per AEC protocol approval.	

Conditions:

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IV. SAFETY AND COMPLIANCE

- The person undertaking this task must ensure all relevant approvals are in place, training has been undertaken, and risk assessments have been performed. If unsure, consult your supervisor.
- Facility protocols should be followed.
- Possible risks include rodent bite injury, needle stick injury, spills, exposure to infectious agents, repetitive task musculoskeletal injury and psychosocial harm.

V. TRAINING CONSIDERATIONS

- All unsupervised animal injections must be performed by appropriately trained personnel who have been deemed to be competent in the procedure.
- Training for injections must be undertaken on models or cadaver animals initially, then live animals which are under anaesthesia.
- For UQBR Training purposes:
 - The AEC approved volume for the project may be administered.**
 - If using the closed injection method, consider performing this injection with dye in cadavers to assess location before starting your experiment.
 - Animals may be recovered from the anaesthesia and held post-procedure for monitoring. Adverse effects may take time to develop and can assist with the assessment of competency.

VI. EQUIPMENT

- PPE *Minimum PPE is gloves and gown; additional PPE may be required based on facility or additional risk e.g. working with infectious animals.*
- Heat source with ongoing monitoring
- Disinfectant*
- 1ml Syringe and Needle (30-25G) **
- Scalpel (if using the open injection method)
- Sharps Container
- Experimental compounds for injection**
- Anaesthetic agents**
- Analgesic agents**
- Light source
- Hair removal items* as per LAB_089 Hair Removal in Rodents
- Ophthalmic lubricant (non-medicated, viscous and pH neutral: eg Refresh "Lacri-lube", Visco-tears gel)
- Sterile surgical instruments (*Including: fine surgical scissors, curved forceps*)
- Surgical consumables (*Including: surgical glue, suture material, sterile cotton tips, gauze*)
- Change Station or Biosafety Cabinet Clean recovery boxes – standard housing boxes including sterile feed, water, appropriate nesting materials (to aid thermal support) and environmental enrichment.

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VII. PREPARATION

1. Check AEC approvals to ensure the correct procedure and personnel are approved for the planned work.**
Variations can occur between procedures listed in this SOP versus what is approved with the animal.
2. Set up equipment items.
There should be no contamination of equipment or substances during this process. Ensure all items to be used have been sterilised in accordance with the appropriate aseptic/clean technique SOP.
3. Turn on Change station or Biosafety Cabinet*
Ensure equipment is operating as required.
4. Wipe surfaces with disinfectant.*

Preparation of Injection Substance

- It is the responsibility of the researcher to fully inform all workers of any risks associated with compounds and materials to be used. This may include lab specific risk assessments and SDS and other OHS obligations.
If substances to be used are experimental or off label (i.e. no Safety Data Sheet is available), the laboratory is responsible for advising workers of all risks involved in the project. This includes risk of performing the procedure as well as the risks associated with animal husbandry such as waste management of cage bedding and cadavers that UQBR staff may be exposed to. Exposure may be acute or chronic.
- Prepare the solution for injection
Remove air bubbles by holding the syringe upright and tapping it or gently moving the plunger back and forth. If injecting cells, a 25G needle is recommended to prevent damage to the cells. If you are injecting cells you may need to keep the syringe on ice to ensure cell viability. A new needle must be used for every animal to maintain sterility between mice and to avoid discomfort from bluntness.

VIII. PROCEDURE

Mammary Fat Pad Injection Procedure

1. Prepare yourself and the workstation as per [LAB_001 Aseptic Technique for Laboratory Animal Surgery](#) or [LAB_002 Clean Technique for Laboratory Animal Surgery](#)
2. Prepare heat pad and clean recovery boxes (e.g. resting on a heat mat). As per [LAB 058 Heating Procedures in Mice and Rats](#).
3. Anaesthetise the animal**
4. Before proceeding, ensure the animal has reached an appropriate depth of anaesthesia/chemical restraint - refer to [LAB_025 Rodent Anaesthesia - Injectable Agents](#) or [LAB_060 Rodent Anaesthesia – Isoflurane](#), whichever is appropriate for the approved form of anaesthesia.
5. Remove hair at the injection/surgical site.
Remove fur from the mid abdomen to just below the 5th teat. As per [LAB_089 Hair Removal in Rodents](#).
6. Apply ophthalmic lubricant to both eyes, using a sterile cotton tip.
7. Prepare the animal for surgery, place into dorsal recumbency.

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8. Use one of the below injection methods.



Figure 1. Animal surgically prepped.

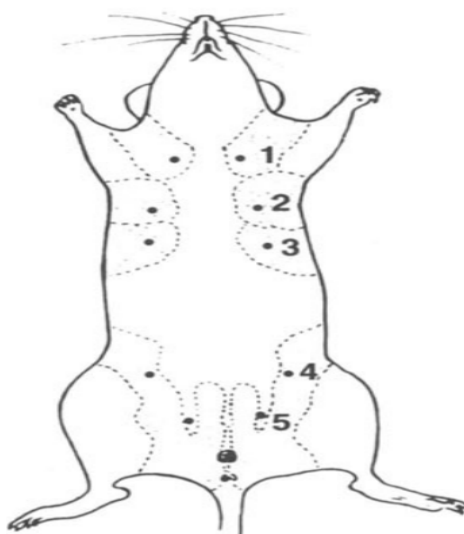


Figure 2. Diagram of mouse mammary gland location, 1. Cervical 2 and 3. Thoracic 4. Abdominal 5. Inguinal
Black dots represent the nipples (ref. Ip & Asch 2012,).

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---	---	--

Closed injection method – Generally a quicker technique with the benefit of not resulting in a surgical wound, however users must be very experienced as there is a risk of injection into the abdomen or subcutaneously next to the mammary tissue.

- Using curved forceps gently grasp the skin above the gland and lift the skin to make a ‘tent’.
- Hold the syringe in your dominant hand with the bevel up and insert it ~2–3 mm deep into the centre of the tented skin, avoiding the nipple and following the direction of the mammary fat pad.
Mammary glands can extend from the underside of the body (belly) up toward the shoulder blades (intrascapular area), and from the sides (flanks) down toward the rear end (perineal region). See figures 3 and 4.
- Release the skin from the forceps to remove any tension on the tissue.
- Inject pre-determined volume slowly**
- Wait 3-5 seconds then slowly and smoothly remove the needle.
This will help prevent leakage. If any leakage still occurs a clean swab can be used to apply gentle pressure.

If leakage is observed, stop and adjust the injection site, as the needle may not be correctly placed. Ensure the skin is clean, dry, and free of cuts or scratches. A small bleb at the site is normal and indicates a successful injection.



Figures 3 and 4 Closed method location for abdominal mammary gland (UQBR 2025)

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---	---	--

Open Injection Method - A direct approach through a small surgical incision. Accuracy can be better than the closed method due to the direct visualisation of the mammary tissue prior to injection. This technique is used for access to the inguinal and abdominal mammary tissue.

- Using a new scalpel blade make a small 2-4mm incision approximately 5mm between teat 4 and 5 to locate the inguinal mammary tissue.
Ensure that the incision is made in a location that minimises tension at the wound site. Tent the skin, then use scissors at a perpendicular angle to make a shallow incision. Avoid cutting too deep to prevent damaging the abdominal muscle.
- Create a small pocket at the incision site using a sterile saline-moistened cotton swab, then gently use forceps to expose the mammary tissue.
The mammary tissue should be a visible shiny white subcutaneous mass outside the peritoneal cavity. To prevent it from drying out, perform the injection promptly or keep the area moist with sterile saline.
- Holding the syringe in your dominant hand, insert the needle and inject the pre-determined volume slowly. Wait 3-5 seconds then slowly and smoothly remove the needle. See Figure 5.
After injection into the mammary tissue, a small bubble should form within it. If leakage occurs, stop immediately and adjust the injection site. Persistent leakage may indicate issues with the injection volume or solution viscosity and should be reviewed.
- Close the wound using the chosen method in the AEC approved project.** Where the incision is only 2-4mm, surgical glue is adequate for closure.



Figure 5 Open injection method- Exposure of mammary gland after incision and being injected.

- Place the animal into a recovery box, maintained on a heat mat until recovered from anaesthesia. If available, recovery boxes may then be placed into a climate controlled, Ventilated Cabinet® for ~12 hours recovery.

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		Page 7 of 7

10. You must clean and disinfect all equipment between each animal.
11. Continuously monitor (in line of sight) all rodents during surgery and throughout the recovery phase until ambulatory and ready to be returned to the home cage. Ongoing monitoring is as described by the approved AEC activity.
12. To confirm successful injection placement, consider the use of labelled cell and imaging. This needs to be approved by the AEC.
13. Ensure the cage card and all records are updated. Use the UQ Surgery - score sheet for mice with surgical wounds where the open method is used.

IX. BIBLIOGRAPHY

1. NHMRC, 2013, Australian code for the care and use of animals for scientific purposes, National Health and Medical Research Council (NHMRC).
2. Ip. M & Asch, 2012, *Methods in Mammary Gland Biology and Breast Cancer Research*. Springer. [Comparative Anatomy and Histology \(Second Edition\) A Mouse, Rat, and Human Atlas](#)

Version #	Reviewing AEC	AEC Review Date	Outcome
2	Anatomical Biosciences AEC, Health Sciences AEC, Molecular Biosciences AEC and Laboratory Biomedicine AEC	16/02/2022 12 months extension approved March 2025	Approved to March 2026
3.2	Laboratory Biomedicine AEC	07/05/2026	Approved to 07/05/2029

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