

Use of ALZET® Osmotic Pumps in Immunodeficient Mouse Models



"The combination of the established SCID mouse model for RA and the long-term osmotic pump-based delivery system for bioactive molecules adds a novel tool for the detailed analysis of RA pathophysiology." (Knedla, A, et al. *Ann Rheum Dis* 2009;68:124-129)

"The comparative experiments (injection and infusion) confirmed that continuous treatments and long-term administration were associated with the best treatment responses." (Tejeda, M, et al. *Anticancer Research* 2006; 26(4B): 3011-3015)

Immunodeficient mouse models have been an integral part of pre-clinical research, and have contributed to significant advances in oncology, transplantation, immunology and stem cell biology research. There are two main species of immunodeficient mice used in research today: SCID and Nude mice. **SCID** (severe combined immunodeficiency)

mice lack the ability to make T or B lymphocytes due to a genetic mutation in chromosome 16. **Nude** mice also undergo a genetic mutation (disruption of the FOXN1 gene) that leads to a lack of, or deteriorated thymus gland and inability to produce T lymphocytes. In addition to inhibiting their immune system, this mutation also causes a lack of hair growth in the animals, hence their name.

Since these species do not exhibit normal immunological responses (e.g., fighting infections or rejection of tumors or transplants), they have been extensively used as biological hosts for tissue transplants since. It is this particular feature that makes these species extremely

valuable in transplantation, oncology, and immunology research.

ALZET Osmotic Pumps have been used extensively in immunodeficient mice since 1980, and over 250 publications are available as evidence of their research value in these species. The automatic operation and small size of ALZET pumps

makes them an ideal infusion system for chronic dosing studies in Nude and SCID mouse models. No researcher intervention is required during infusion, and animal handling is kept to a minimum to reduce the risk of infection and stress.



Nevertheless, since SCID and Nude mice are more delicate than normal mice, there are inherent concerns of using them in research studies involving experimental procedures, such as ALZET pump implantations. This technical sheet is intended to serve as a guide for successful use of ALZET pumps in SCID and Nude mice.

COMMON CONCERNS, AND SOLUTIONS, WHEN USING ALZET OSMOTIC PUMPS WITH IMMUNODEFICIENT MICE:

- **Pump size is too large.** *The smallest ALZET pump models (100X series) are only 1.5 cm in length x 0.6 cm in diameter. These pumps can be implanted subcutaneously in animals as small as 10 grams. They have widely been used in very small rodents (over 600 references), including neonatal rats and small mice.*
- **Procedure is too invasive.** *The subcutaneous ALZET pump implantation is minimally invasive, and it is relatively quick and easy to perform. In fact, this procedure can typically be completed within a few minutes under anesthesia.*
- **Risk of infection.** *The ALZET pumps are sterilized via irradiation prior to shipping. Hence there should be no added risk of infection when using our products. However, when working with immunodeficient rodents, the need for maintaining aseptic technique is paramount for preventing infection.*
- **Thin and delicate skin.** *Nude mice in particular have very thin skin, which (compared to normal mice) can be easily damaged by the presence of a subcutaneous implant. Tissue necrosis can occur due to constant tissue rubbing within a tight subcutaneous compartment. To minimize irritation and pressure necrosis of the skin overlying the pump, be sure that the subcutaneous tunnel is at least 1 cm longer than the pump. Also, minimize drug/solvent irritation of the incision by inserting pumps with the exit port facing away from the incision.*

THE FOLLOWING ARE ADDITIONAL CONSIDERATIONS FOR USING THE ALZET OSMOTIC PUMPS WITH IMMUNODEFICIENT MICE:

Pre-Surgical Considerations

Surgical Room

- Lab benches in front of open windows, next to doors, or in similar locations where air currents and dust are difficult to control should be avoided.
- An area in or in front of an exhaust hood should not be used; a high efficiency particulate absorbent (HEPA)-filtered hood, glove box, or plastic bubble is acceptable.

- Personnel who enter animal rooms to handle nude mice should be careful not to touch any other animals or equipment. If they must handle other animals, they should start work with nude mice before other daily routine work.

Surgical Area

- Before surgery, lab bench or table should be cleaned and disinfected; quaternary ammonium disinfectants or 70% alcohol are good choices.
- Establish special areas and equipment for use with immunodeficient mice only.
- Rodent surgical area can be a room or part of a room that is easily sanitized and not used for other activities when rodent surgery is in progress.
- The area should be subdivided into specific places for rodents awaiting or recovering from surgery, preparing rodents for surgery, and performing surgery. This reduces potential contamination by fur, feces, and bedding.
- All injections, surgery, and pump preparation should be performed steriley in a laminar-air-flow room or under a laminar-air-flow hood by steriley gowned, masked, and gloved operators and assistants.
- A cork board, plastic tray, or a few paper towels can be placed under the rodent to minimize heat transfer during short procedures.
- Temperature-controlled small water 'blanket' should be placed under the rodent during prolonged surgical procedures to prevent hypothermia.

Instrumentation

- Autoclavable tip guards for surgical instruments should be used.
- Special instrument trays with rows of soft plastic fingers can prevent damage of delicate instruments.
- Check the tips of delicate instruments--preferably under a microscope--to make sure they are not damaged or dull, and check grooves to verify that no blood or tissue remains.
- Do not use damaged or dull instruments.
- Steam or dry heat is preferred to sterilize surgical instruments; sterility should be verified through periodic use of biological indicators.
- Instrument packs sterilized by ethylene oxide must be aerated to remove residual gas
- Some chemical sterilizers can irritate tissue.
- Use clear and lightweight surgical drapes; opaque disposable paper or cloth drapes make it difficult to monitor the respiratory rate of small rodents.
- Anesthesia equipment is the same as for normal mice.

Surgical Considerations

Animal Preparation

- ALZET pumps are suitable for subcutaneous (SC) and intraperitoneal (IP) implantation in mice according to the animal size guidelines described below.

MINIMUM ANIMAL WEIGHTS

Implantation Route	Pump Models	Pump Models
	1003D, 1007D, 1002, and 1004	2001D, 2001, 2002, 2004, and 2006
Subcutaneous	10+ gram mice	20+ gram mice
Intraperitoneal	20+ gram mice	Via catheter placement

- It is important to remove the fur over the incision site, and to decontaminate the skin.

- Decontamination of skin should be accomplished without soaking the body of the rodent.
- Alternatives for removing fur include: plucking, clipping, shaving, or, in selected instances, depilatories.
- After fur is removed, skin must be cleansed and disinfected; begin at the incision site and work outward in circles of increasing diameter.

Surgery

The skin of Nude mice may have less mechanical strength than normal skin, requiring careful suturing using a finer needle and more stitches per unit length. Tissue adhesive may also be preferable depending on your institution's guidelines.

In Between Surgeries

- Contamination can be reduced by segregating surgical instruments according to function.
- Use glass bead sterilizers, sterilize unwrapped instruments quickly, and cool instruments on sterile surface before use.
- In repetitive rodent surgery, wiping instruments with 70% alcohol and a sterile swab between rodents can reduce bacterial contamination on a short-term basis.
- Even with the techniques mentioned above, a sterile instrument pack should be used after every 4 or 5 individual rodents.

Post-Operative Considerations

- An electric light (50-75 W bulb) suspended over one end of the cage is a simple heat source for rodents recovering from anesthesia.
- After use, instruments should be soaked in lukewarm water to remove blood and tissue, washed with free rinsing neutral pH detergent, rinsed thoroughly, and air dried.
- Water and food do not need to be withheld. The inability of mice and rats to vomit prevents regurgitation of stomach content.

Additional Tips/Methods Extracted from Recent Publications

Mice were “allowed to acclimate to local conditions for at least 1 week” P8817 **Dings, R.P.M, et al. Cancer Letters 2008;265:270-280**

“Alzet pumps were aseptically filled with the specific solutions, and implanted subcutaneously into the left lateral flank (opposite to the tumor) of the mice under anesthesia (ketamine 100 mg/kg and xylazine 3 mg/kg), and the incision was closed by wound clips.” **Dings, R.P.M, et al. Cancer Letters 2008;265:270-280**

“Animals were anesthetized with 2.5% isoflurane during imaging (BLI).” **Zhang, Y.M, et al. Cancer Research 2007; 67(19):9389-9397**

“Six- to 7-week-old nude mice were housed in a pathogen-free room in microisolators with autoclaved bedding and fed autoclaved rodent chow and water.” **Song, P, et al. Cancer Research 2007;67(8):3936-3944**

“During the infusion period, the animals maintained their body weight as well as food and water consumption and showed no evidence of reduced motor function.” “The mice (athymic) were anesthetized by inhalation with 1.5% isoflurane **Menon, J, et al. Cancer Research 2007; 67(6):2809-2815**

“After connecting the catheter tube with the cannula, the skin was closed with 6-0 silk sutures.” **Kawakami, K, et al. Journal of Immunotherapy 2005; 28(3):193-202**

Sources

“Guide for the Care and Use of the Nude (Thymus-Deficient) Mouse in Biomedical Research”, A Report of the Committee on Care and Use of the “Nude” Mouse , Institute of Laboratory Animal Resources Assembly of Life Sciences, National Research Council. Reprinted from ILAR NEWS , Vol XIX (2), 1976.

Cunliffe-Beamer, Terrie L., DVM, MS. "Applying Principles of Aseptic Surgery to Rodents." AWIC Newsletter, April-June 1993, Vol. 4, No. 2.

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"Anesthesia and Surgery of Laboratory Animals," William J. White, V.M.D., M.S. and Karl J. Field, D.V.M. in Veterinary Clinics of North America: Small Animal Practice, Vol 17(5), 989-1017. [general information on surgical and anesthetic techniques on laboratory animals (including rabbits, guinea pigs, rats and mice)].