WORLD
PRECISION
INSTRUMENTS

INSTRUCTION MANUAL

TheraCyte

(503395, 503396, 503397) Cell Encapsulation Device

Syringe Loading

Centrifuge Loading

Implanting TheraCyte Device

www.wpiinc.com

030421

CONTENTS

SYRINGE LOADING METHOD

This procedure applies to researchers using the Theracyte device.

SCOPE

This procedure describes the loading of the TheraCyte, Inc.'s ported immunoisolation devices (TheraCyte® system) with cells or tissue using a hamilton syringe. This loading method applies to the 4.5 µl, 20 µl and 40.0 µl ported immunoisolation devices used in the TheraCyte® system.

BACKGROUND/TECHNOLOGY

The TheraCyte® system includes an implantable membrane device that contains cells or tissues that secrete therapeutic products. The outer membrane will promote vascular structures to form at the membrane-tissue interface and the inner membrane will prevent host cells from infiltrating the transplanted material. Devices are manufactured at a TheraCyte facility. Final preparation, tissue loading, closure and implantation are completed at the site of use.

This loading method has been used to load MSU1.2 a human fibroblast cell line into ported immunoisolation devices. The following cell concentrations and loading volumes were noted:

For 40 µl devices: Approximately 2.0 x 10^7 MSU1.2 cells in a final volume of 40 µl are loaded into the 40 µl device.
For 20 µl devices: Approximately 1.0 x 10^7 MSU1.2 cells in a final volume of 20 µl are loaded into the 20 µl device.
For 4.5 µl devices: Approximately 2.5 x 10^6 MSU1.2 cells in a final volume of 5 µl are loaded into the 4.5 µl device.

NOTE: Less dense cell concentrations may be used. For example, 2.0 x 10^7 cells in a final volume of 60 µl may be used for the 40 µl device, or 1.0 x 10^7 cells in a final volume of 30 µl may be used for the 20 µl device. Also if the cell being used is proliferative then the cell number will increase in vivo.

It should be noted that the following loading method may not work effectively in all situations depending on the characteristics of the cell used.

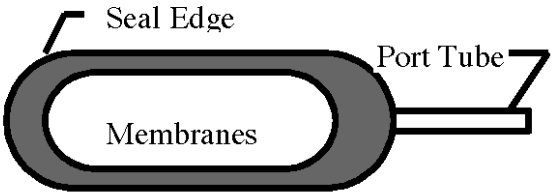
MATERIALS/EQUIPMENT

TheraCyte® ported immunoisolation devices
Ethanol, 95 % and 70 %, filtered (0.2 µm)
Sterile saline (0.9 % NaCl)
Hamilton Syringe, sterile, sizes appropriate to volumes loaded
Blunted 22 G Hamilton needle, sterile
Medical Grade Silicone Tubing, sterile
Sterile Luer Stub Adapters, 15 gauge, n = 1
Silastic® Medical Adhesive Silicone Type A, sterile
3 cc sterile syringe with threaded male Luer connector, n = 1
Sterile atraumatic forceps, n = 2
Sterile scalpel blade, size 10
Centrifuge tubes, 15 ml or equivalent
Sterile gauze

PREPARATION PROCEDURES

DEVICE HANDLING

Use atraumatic sterile forceps or equivalent to handle device. Touch device only by port tube or seal edge to avoid contaminating device or breaching membranes. Membrane contact may cause damage to device. See device figure below.



Some of the PTFE membranes are hydrophobic (just like Gortex fabric) and will repel water and not let diffusion occur. Therefore, the membranes are wetted with alcohol and then washed and left in saline prior to use. After wetting it is important not to let the membranes dry out or the wetting process will have to be repeated.

DEVICE WETTING AND STERILIZATION

1. In a sterile environment, open pouch containing device.

2. Membranes are wetted by submerging entire device into sterile 15 ml centrifuge tube or sterile culture dish filled with filtered 95 % ethanol until device membranes appear wetted or transparent.

   NOTE: If devices are received sterile, skip step #3.

3. Submerge entire wetted device into sterile 15 ml centrifuge tube or sterile culture dish filled with filtered 70% ethanol. Soak devices for at least two hours to sterilize, preferably overnight.

   It is recommended to sterilize a few extra devices for each experiment.

4. Submerge entire wetted, sterile device(s) into sterile 15 ml centrifuge tube or sterile culture dish containing sterile saline.

5. Rinse device(s) by gently shaking centrifuge tube or dish, then soak for at least 20 minutes.

6. Repeat steps 4 and 5 two times for a total of three 20 minute rinses with fresh sterile saline.

7. Store wetted, sterile devices in sterile saline or any other buffered saline/media solutions until they are loaded.

DEVICE LOADING PREPARATION (In a sterile environment)

1. Place all loading materials in sterile work area.

2. Fill 3 cc syringe with sterile silicone adhesive by removing plunger and docking adhesive tube to proximal end of syringe. Dispense approximately 1.5 cc of adhesive into each syringe barrel. Keeping sterile, close adhesive tube and store refrigerator or freezer for next use. If kept closed and used sterile, this silicone should be good for multiple experiments.

3. Reconnect 3 cc syringe plunger. Slowly insert plunger until all air is expelled from syringe. Visually inspect syringe to make sure adhesive is free of air bubbles.

4. Connect Luer stub adapter and prime with the adhesive by pushing some adhesive through Luer stub adapter. Wipe off excess adhesive with sterile gauze. Adhesive containing syringe will be referred to as sealing syringe.

   NOTE: Fill syringes with medical adhesive within 15 minutes of initial use. Adhesive may remain in syringe without curing for at least one hour.

5. Assemble a sterile Hamilton syringe *(See page 8 for sterilzation method)* by wetting Teflon® plunger and inserting into glass barrel.

   NOTE: Do note attempt to assemble plunger into syringe barrel while dry since damage to Teflon® plunger tip may occur.

6. Wet needle and Teflon® o-ring washer by dipping in sterile saline and connecting to distal end of glass barrel.

7. Prime Hamilton syringe and needle using plunger to repeatedly aspirate and expel media from needle until no air bubbles are visually detected in glass syringe barrel.

LOADING PROCEDURE (In a sterile environment):

1. Draw up the following cell suspension and media amounts using a sterile Hamilton syringe fitted with a sterile blunted 22 G needle:
   a) For a 40 µl device--use a 100 µl syringe and draw up 20 µl of media, then 80 µl of cell suspension, total syringe volume is 100 µl;
   b) For a 20 µl device--use a 100 µl syringe and draw up 20 µl of media, then 40 µl of cell suspension, total syringe volume is 70 µl;
   c) For a 4.5 µl device--use a 25 µl syringe and draw up 10 µl of media, then 15 µl cell suspension, total syringe volume is 25 µl.

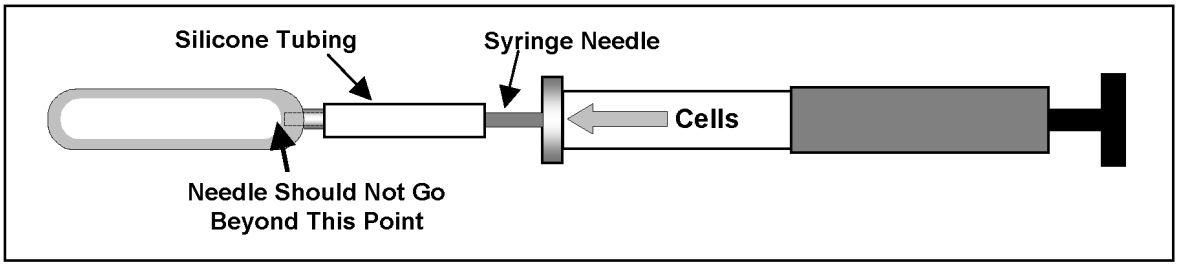
   NOTE: Vortex or mix cells before loading into syringe to maintain consistent cell suspension.

2. There are two alternatives for step 2. (A) is the preferred but use (B) if tissues fail to distribute evenly in the device. Be aware membrane damage can occur with step (B).

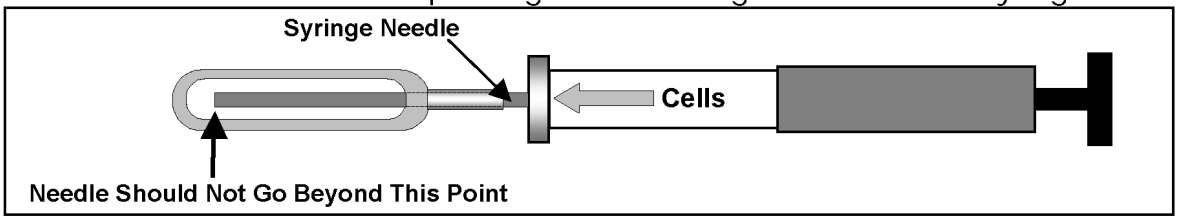
A. 1. Position sterile silicone tubing piece, with the clear end over the port tube until silicone tubing is approximately 0.2 - 0.3 inches from device edge.

   2 Insert blunted 22 G needle of Hamilton syringe through device port and just into welded area of device. Do not progress further into the lumen.

   NOTE: The needle tip should not enter into the device or touch the device lumen. Hold port tubing with sterile forceps to aid in needle insertion. See figure below.



B. Insert blunted 22 G needle of Hamilton syringe through device port and into device lumen, rotating needle to assist insertion. Stop when the needle is approximately 3/4 into the device. Expel approximately 50 % of syringe volume, then slowly withdraw the needle while dispensing the remaining contents of the syringe.

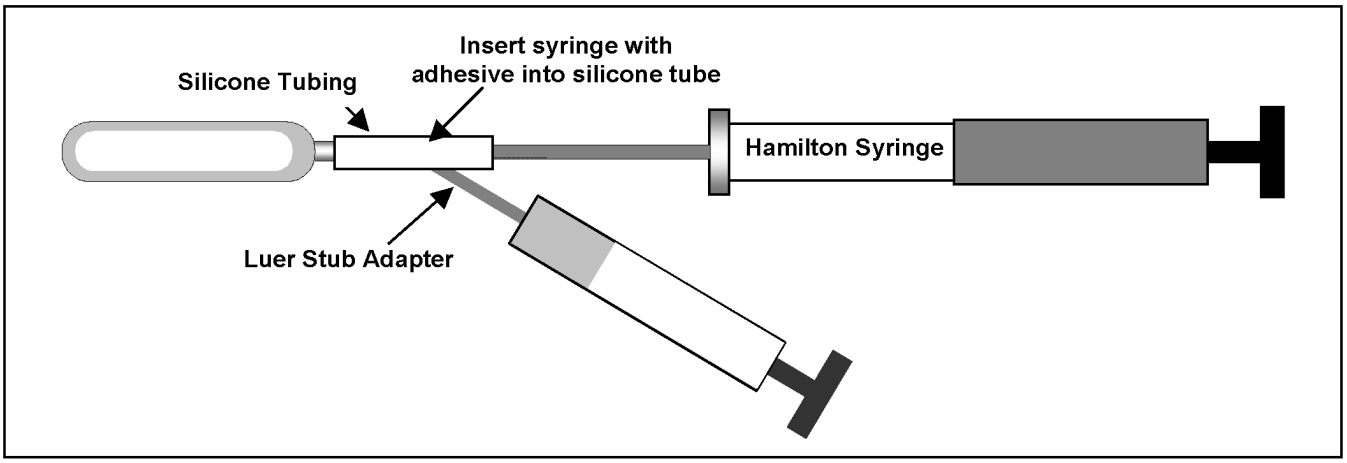


3. Keeping syringe stationary, slowly expel syringe contents into port tubing and device.
   NOTE: Media and cells may travel up device port.

4. Carefully withdraw syringe needle to insure cell droplets do not escape out of tubing.

DEVICE SEALING

1. Insert luer stub adapter of sealing syringe to silicone tubing containing cells. Use forceps to hold silicone tubing to make an air free connection.



2. Slowly inject silicone adhesive into silicone tubing and port tube until silicone adhesive reaches device inner lumen.
   NOTE: For 40 µl devices, you will feel resistance to silicone flow upon reaching device inner lumen. The silicone adhesive volume is approximately 0.05 cc.

3. Cut port tube below the silicone tubing using a sterile scalpel blade or sterile scissors. If possible, keep sealing syringe connected to silicone tubing when cutting.

4. Inspect for presence of silicone adhesive and trim port tube at 90 angle to desired size for implantation. We recommend cutting device port no shorter than 4 millimeters.

5. Observe port tubing to insure that silicone does not extrude out of port by more than one millimeter.

Hamilton Needle Cleaning and Inspection

SCOPE

The following procedure define the method for the cleaning and inspection of blunted Hamilton® needles used in loading ported immunoisolation devices.

BACKGROUND

The needles are obtained blunted (at 90 from the needle shaft) from the Hamilton company. Following each device loading, the needles are cleaned and inspected.

MATERIALS

Needles, Hamilton Company, 22 gauge
Hamilton Cleaning Solution Concentrate, # 18310
Hamilton Cleaning Wire, 12 mil, # 18304
Branson 2200 Ultrasonic Cleaner # L7633, or equivalent
Microscope, Wild, Heerbrugg, Asset # RLS 20324, or equivalent
Imperial 4 Microprocessor Oven, Asset # RLS 21292, or equivalent
Light Source, Fiber-Lite High Intensity Illuminator Series 180, Dolan-Jenner Industries
Deionized MilliQ® water (dH_2O)

METHODS

Cleaning

1. The cleaning wire is inserted through the end of the needle, pushed through and removed through the needle tip. The wire is not pushed back through the end of the needle.
2. The needles are placed in the ultrasonic cleaner. A 1:4 (solution : dH_2O) dilution of the cleaning solution concentrate is added to completely cover the needles. The ultrasonic cleaner is set for five minutes and turned on.
3. Following cleaning, the solution is removed and replaced with dH_2O. The needles are allowed to rinse for five minutes under sonication.
4. After the needles are rinsed, they are immersed in filtered (0.22µm) 70 % EtOH and gently agitated for approximately 30 seconds to remove the water.
5. The needles are removed from the EtOH and deposited on a clean absorbent surface and then transferred to a clean tray or a sheet of aluminum foil. The tray is placed in the oven set at a temperature no greater than 95 C. The needles are allowed to dry for at least 10 minutes.

NEEDLE INSPECTION

1. Visually inspect entire needle under the microscope (25 x power) for imperfections, burrs and miscellaneous deformities. Use the light source to insure adequate lighting.
2. Inspect the milled end of needle under both 25 and 50x magnification. Look for metal shavings, burrs, flat sides, rough edges from unfinished grinding, and uniform roundness. The stem of the needle should be relatively smooth away from the milled area.
3. Observe milled area from a top and side view (both 25 and 50 x mag.). Rotate needle 360 while viewing. Make sure the needle tip does not extend more than 60 µm from all sides while inspecting at 50 x magnification.
4. The needle should be accepted for use if it has uniform roundness and no inconsistent markings.
5. The needles that pass inspection are stored and sterilized prior to use.

Sterilization of Hamilton Syringes and Needles

SCOPE

The following procedure describes the method for sterilizing Hamilton® syringes and needles prior to their use in loading Baxter Gene Therapy Unit's ported immunoisolation device (TheraCyte™ system).

BACKGROUND

The syringes and needles are obtained from the Hamilton company. The needles are cleaned and inspected for quality. The needles that pass inspection are then sterilized prior to use in device loading. Following each device loading procedure, the needles are cleaned and inspected.

MATERIALS

Syringe, Hamilton Company, 10, 25, 100, or 250 µl volumes
Pyrex Glass Test Tubes with Screw-on caps
Sterilization Pouches
Autoclave-Equipped with an Isothermic Cycle

METHODS

1. Remove syringe barrel from plunger. Place syringe plunger into glass test tube, Teflon® tip inside first.
2. Place syringe barrel and clean needle inside test tube.

NOTE: If test tube too small, place needle and barrel in separate test tube.

3. Put test tube(s) containing syringe and needle into sterilization pouch and seal.
4. Autoclave syringe using isothermal cycle, no greater than 95 °C, for no longer than 15 minutes.
5. In a sterile environment, assemble Hamilton syringe by wetting Teflon® plunger with sterile saline and inserting into glass barrel.

NOTE: Do not attempt to assemble plunger into syringe barrel while dry since damage to Teflon® plunger may occur.

6. Wet needle and Teflon® o-ring washer by dipping in sterile saline and connecting to distal end of glass barrel.
7. Prime Hamilton syringe and needle using plunger to repeatedly aspirate and expel media from needle until no air bubbles are visually detected in glass syringe barrel.

Ordering Information for TheraCyte Loading Accessories

Hamilton Syringes & Needles

Syringes (Gastight, removable needle)

Model No.	Volume	Hamilton Cat. No.	Price at 9/1/2000
1802RN	25µl	84880	$76
1805RN	50µl	84883	$78
1810RN	100µl	84886	$80
1825RN	250µl	84889	$85

Needles (Gastight, removable needle syringes)

Description: RN Needle Rheodyne (22/2"/3)S 3/pk
22G, 2" for 25, 50, 100µl syringes,
Outer Diameter, 0.028"/0.72mm, Inner Diameter, 0.016"/0.41mm
Cat. No. 80468, $13

Description: RN Needle Rheodyne (22/2"/3)L 3/pk
22G, 2" for 250µl syringes, Outer Diameter, 0.028"/0.72mm, Inner Diameter, 0.016"/0.41mm
Cat. No. 80763, $13

Phone or fax order directly to Hamilton Company.
Toll-Free Order Hotline in the U.S.A.: 1-888-525-2123

Dow Corning Medical Adhesive Type A

One-component, translucent silicone material for bonding synthetics, metals or silicone elastomers. Contains no solvents. Cures at room temperature on exposure to or moisture. Small amounts of acetic acid vapor liberated while curing. Skin-over time, 5 minutes. Shelf life prolonged by refrigerator/freezer storage.

Distributed by Factor II, Inc. phone: 520-537-8387/520-537-8688, Fax: 520-537-8066
Cat No. A-100-S, 6gm tube (sterile), $17.95
Note: Requires a letter of compliance to be signed, call Factor II, Inc. for details.

CENTRIFUGATION LOADING METHOD

This procedure describes a method of preparing TheraCyte® Devices and loading with islets or cells

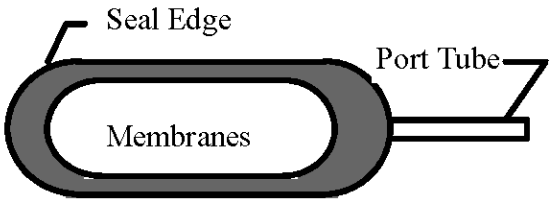
MATERIALS

TheraCyte® devices
DPBS, sterile, Sigma, D-5527,
Centrifuge
Medical Grade Silicone Tubing, 0.062" I.D. x 0.125" O.D., Baxter S/P, sterile, cut into 0.5" pieces
Ethanol, 100%, filtered (0.2µm), Spectrum
Ethanol, 70%, filtered (0.2µm), Spectrum
Silastic® Medical Adhesive Silicone Type A, sterile, Dow Corning
3cc sterile syringe with threaded male Luer connector
Sharp 20 gauge needle, sterile
Pipette tips, 200µl

PROCEDURE

DEVICE HANDLING

Use atraumatic sterile forceps or equivalent to handle device. Touch device only by port tube or seal edge to avoid contaminating device or breaching membranes. Membrane contact may cause damage to device. See device figure below.



DEVICE WETTING AND STERILIZATION

1. In a sterile environment, open pouch containing device.

2. Membranes are wetted by submerging entire device into sterile 15ml centrifuge tube or sterile culture dish filled with filtered 95-100% ethanol until device membranes appear wetted or transparent.

NOTE: If devices are received sterile, skip step #3.

3. Submerge entire wetted device into sterile 15 ml centrifuge tube or sterile culture dish filled with filtered 70% ethanol. Soak device for at least two hours to sterilize.

NOTE: It is recommended to sterilize a few extra devices for each experiment.

4. Submerge entire wetted, sterile device into sterile 15 ml centrifuge tube or sterile culture dish filled with sterile saline.

5. Rinse device by gently shaking centrifuge tube or dish, then soak for at least 20 minutes.

6. Repeat steps 4 and 5 two times for a total of three 20 minute rinses with fresh sterile saline.

7. Store wetted, sterile devices in sterile saline until they are loaded.

Device Loading:

A 200µl pipette tip was cut so that the inner diameter of the tip was approximately 0.035", the same as the device port. The pipette tip was connected to the device port using a 0.5" piece of silicone tubing (0.062" x 0.125"). This assembly was placed in a 15 ml test tube filled with enough saline to cover the top of the device. Figure 1 illustrates the assembled components used for centrifuge loading. Islets or cells are then loaded into the connected tip (holds approximately 250µl) and the test tube was placed in the centrifuge and spun for 1-2 minutes at 1000 rpm (approx. 200 g). The tissues will have entered the device. Keep the port and pipette tip upright in the centrifuge tube. The device is now ready for sealing.

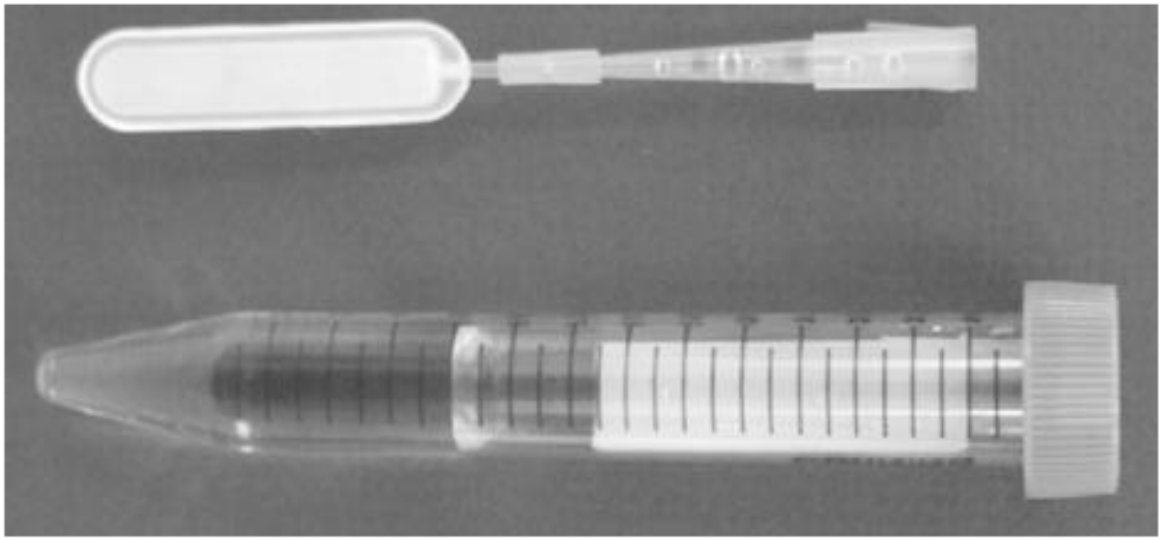


Figure 1. Assembled components for centrifuge loading.

Device Sealing:

Steps 1-4, syringe preparation, can be prior or during device loading.

1. Create sterile work area within a laminar flow hood.

2. Fill 3 cc syringe with silicone adhesive by removing plunger and docking adhesive tube to proximal end of syringe. Dispense approximately 1.5 cc of adhesive into each syringe barrel.

3. Reconnect 3 cc syringe plunger. Slowly insert plunger until all air is expelled from syringe. Visually inspect syringe to make sure adhesive is free of air bubbles.

4. Connect Sterile 20 guage needle and prime with the adhesive by pushing some adhesive through the needle. Wipe off excess adhesive with sterile gauze. Adhesive containing syringe will be referred to as sealing syringe.

NOTE: Fill syringes with medical adhesive within 15 minutes of initial use. Adhesive may remain in syringe without curing for a maximum of one hour.

5. Bend the silicone tubing at the end of the pipette tip . Pierce the silicone tubing and dock the needle onto the device port. The 20 guage needle will create a tight fit onto the device port

6. Injection the silicone at a rate no greater than 100 μL/min.

7. Device filling and sealing is completed when the silicone adhesive has passed the device duckbill and has begun to enter the device lumen.

8. Cut the port leaving approximately 5-8 mm of the port remaining on the device. Remove the excess port and pipette tip from the sealing syringe and discard the excess port and pipette tip.

9. Visually inspect the port for proper placement of silicone adhesive.

DEVICE IMPLANTATION

PREPARATION

1. Set up sterile surgical field that includes a petri dish filled with saline, and one filled with iodine solution.

2. Fill a 1 cc syringe with vetting glue by docking a 16 gauge needle to the syringe and drawing the glue into the syringe. Remove the 16 gauge needle and replace with a 26 gauge needle for application.

3. Prepare the anesthetic cocktail:
   MICE: 0.75 cc xylazine: 1.0 cc ketamine: 4 cc saline
   Anesthetize the mice with a dose of approximately 0.15 cc cockta il per 20-30 gram mouse, administered interperitoneally.
   RATS: 0.65 cc ketamine: 0.25 cc xylazine
   Anesthetize the rats with a dose of approximately 0.9cc cocktail per kg body weight, administered intramuscularly.

4. Apply eye lubricant to the eyes of anesthetized animal to prevent them from drying out.

FAT-PAD IMPLANT

1. Swab the ventral abdominal area with iodine solution.

2. Make a small skin incision in the lower abdominal region at the midline with the scissors (1 -2 cm for mice, 2-4 cm for rats). Make a similarly sized incision through the abdominal muscle wall.

NOTE: Use a tissue forceps to hold the abdominal wall away from the internal organs while cutting.

3. Remove the epidydimal or ovarian fat pads from the peritoneum and place on a gauze pad wetted in saline.

4. MICE: Place the device on one fat pad with the port pointing cranially. Spread the second fat pad across the top of the device, and secure the device with small amounts of vetting glue.

   RATS: Place a device on one fa t pad with the port pointing cranially and cover with the remaining portion of the fat pad, securing the device with small amounts of vetting glue. Repeat for the second fat pad.

NOTE: Be sure to keep the fat pads moist by swabbing with saline as needed.

5. Return the fat pads containing the device(s) to the peritoneum.

6. MICE: Close the incision by holding the abdominal muscle and the skin from both sides together with tissue forceps and secure with autoclips.

   RATS: Close the incision in the ab dominal muscle wall by suturing using a running cross stitch. Secure the skin with autoclips.

7. Swab the entire abdominal area again with iodine solution.

SUBCUTANEOUS IMPLANT

1. Swab the dorsal area with iodine solution.

2. Make a small incision just lateral to the spine (1 -2 cm for mice, 2-4 cm for rats).

3. Create a pocket between the skin and muscle fascia by blunt dissection.

4. Place the device within the pocket with the port pointing cranially.

5. Close the incision with autoclips.

6. Swab the area with iodine solution.

METHOD FOR IMPLANTING THERACYTE DEVICES IN MICE AND RATS

The two most common places that devices have been be implanted are in the epidydimal (males) or ovarian (females) fat pads or su bcutaneously (ventral or dorsal). With dorsal subcutaneous implants animals are housed separately, but with ventral subcutaneous implants animals can be housed together. The number of devices implanted varies with device size. Maximum recommended numbers are:

For Mice

Device	Peritoneal Fat Pad	Dorsal Subcutaneous	Ventral Subcutaneous
4.5 µl	2 *(1 left pad and 1 right pad)*	2	2
20 µl	1 *(between the two fat pads)*	1	1
40 µl	Not Recommended	Not Recommended	Not Recommended

For Rats

Device	Peritoneal Fat Pad	Dorsal Subcutaneous	Ventral Subcutaneous
4.5 µl	2 *(1 left pad and 1 right pad)*	6	6
20 µl	2 *(1 left pad and 1 right pad)*	4	4
40 µl	2 *(1 left pad and 1 right pad)*	2	2

Below is the currently recommended procedure for device implantation into mice and rats.

MATERIALS/EQUIPMENT

Loaded TheraCyte® ported immunoisolation devices
Iodine Solution, Betadine
Sterile saline (0.9 % NaCl)
Xylazine (Rugby Laboratories, Rockville Center, NY)
Ketamine (Fort Dodge Laboratories, Fort Dodge IA)
Sterile atraumatic forceps, n = 2-4
Sterile Surgical Scissors, n=2
Sterile scalpel blade, size 10
Sterile surgical field (Barrier, sterile field, Johnson & Johnson, Medical Inc.)
Sterile gauze
Petri dishes (10cm)
Eye lubricant.

PREPARATION

1. Set up sterile surgical field tha t includes a petri dish filled with saline, and one filled with iodine solution.

3. Prepare the anesthetic cocktail:

   MICE: 0.75 cc xylazine: 1.0 cc ketamine: 4 cc saline.
   Anesthetize the mice with a dose of approximately 0.15 cc cocktail per 20-30 gram mouse, administered intraperitoneally.

   RATS: 0.65 cc ketamine: 0.25 cc xylazine
   Anesthetize the rats with a dose of approximately 0.9cc cocktail per kg body weight, administered intramuscularly.

4. Apply eye lubricant to the eyes of anesthetized animal to prevent them from drying out.

FAT-PAD IMPLANT

1. Swab the ventral abdominal area with iodine solution.

2. Make a small skin incision in the lower abdominal region at the midline with the scissors (1-2 cm for **mice**, 2-4 cm for **rats**). Make a similarly sized incision through the abdominal muscle wall.

   NOTE: Use a tissue forceps to hold the abdominal wall away from the internal organs while cutting.

3. Remove the epidydimal or ovarian fat pads from the peritoneum and place on a gauze pad wetted in saline.

4. **MICE:** Place the device on one fat pad with the port pointing cranially. Spread the second fat pad across the top of the device, and secure the device with very small amounts of vetting glue placed on the sealed edge of the device, careful not get glue on the (functional) center of the device.

   RATS: Place a device on one fat pad with the port pointing cranially and cover with the remaining portion of the fat pad, securing the device with small amounts of vetting glue. Repeat for the second fat pad.

   NOTE: Be sure to keep the fat pads moist by swabbing with saline as needed.

5. Return the fat pads containing the device(s) to the peritoneum.

6. **MICE:** Close the incision by holding the abdominal muscle and the skin from both sides together with tissue forceps and secure with autoclips.

   RATS: Close the incision in the abdominal muscle wall by suturing using a running cross stitch. Secure the skin with autoclips.

7. Swab the entire abdominal area again with iodine solution.

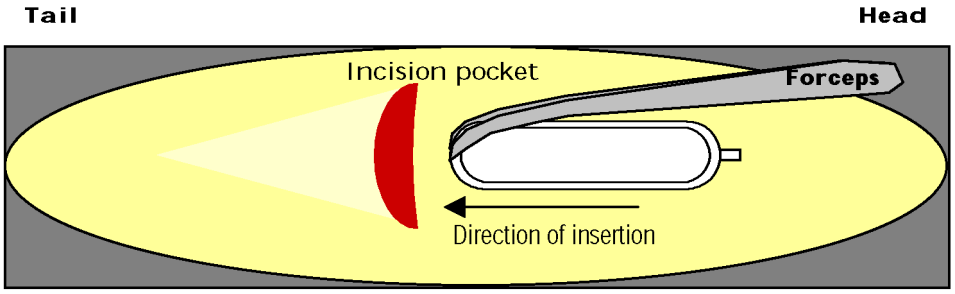
SUBCUTANEOUS IMPLANT

1. Swab the dorsal area with iodine solution.

2. Make a small incision just lateral to the spine (1-2 cm for **mice**, 2-4 cm for **rats**).

3. Create a pocket between the skin and muscle fascia by blunt dissection.

4. Pick up the device, with curve-tipped forceps, from the tail of the device (see diagram below). The device can sit between each arm of the forceps as long as they don't touch (crush) the center of the device. The outer mesh of the device is very "sticky" so try to place the device in the pocket with minimal host contact until the device is situated in the desired location.



Note: Once in position, it is very difficult to re-position the device.

5. Place the device within the pocket with the port pointing cranially and retract forceps.

6. Close the incision with autoclips.

7. Swab the area with iodine solution.

8. Place animal in Housing Box which is preferably on a warming pad until animal awakens.

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