

SOP 24 In Vivo Injection Protocols

Sub-Cutaneous Mouse Injection

Materials

- a. 1mL syringe with 18g 1 ½" needle tip containing solution of interest
 - i. Use a smaller gauge needle when injecting fluids subcutaneously to reduce the discomfort and stress put on the animal
- b. Bucket + ice
- c. Sharps container
- d. Sterile Cages
- e. Alcohol Swab
- f. Matrigel
- g. RPMI (without FBS or additives)

Preparing the Needle

- 1) Prepare 10-50e⁶ strained cells/tumor pieces in 100uL of sterile RPMI media and 100uL cold matrigel per mouse
- 2) Draw solution into tip-less 1mL syringe
- 3) Place 18g needle tip onto syringe and place on ice

Delivery of Sample

- 1) Select strain and number of mice needed
 - i. Nudes (Nu/Nu) are used for subcutaneous tumor models
 - ii. NSG can be used, too, but due to cost concerns, Nude mice should be selected first when available
- 2) Prepare a sterile cage set-up
- 3) Remove sample from ice bucket and uncap the needle
- 4) Using an alcohol swab, clean the area between the animal's shoulders thoroughly
- 5) Using your non-dominant hand, grab the animal between the ears and create a "tent" in the scruff.
 - iii. Certain personnel feel that using the dominant hand is easier; this is a matter of preference as long as the grip feels secure and restrains the animal
- 6) Insert the needle into the loose scruff using extreme caution not to stab your own fingers
- 7) Depress the plunger slowly ensuring the sample is deposited in the "tent" of the skin (between the shoulder blades)
 - iv. Max dose 200uL
- 8) Dispose of needle in sharps container and DO NOT RECAP
- 9) Place the animal back in the cage and observe for complications

Monitoring

Watch for weight fluctuations, tumor size reaching 1500mm³ and general health deviations. Refer to your IACUC protocol for questions.

Tail Vein Mouse Injection

Materials

- a. Insulin needle (28G ½”) containing solution of interest
 - i. Do NOT use an insulin needle even though the gauge size is correct; the length of the needle is not approved and increases the chances of improper placement and organ damage
- b. Bucket + ice
- c. Sharps container
- d. Sterile Cages
- e. Tail warming machine (BrainTree)
- f. Alcohol swabs

Preparing the Needle

- 1) Re-suspend $2-3 \times 10^6$ cells in chilled 1640-RPMI at 200uL/mouse being injected
- 2) Draw solution into insulin needle and place on ice

Delivery of Sample

- 1) Select strain and number of mice needed
 - ii. NSG mice are ideal for intravenous injections
 - iii. NOD/SCID can be used, but they must be irradiated prior to injection
- 2) Prepare a sterile cage set-up
- 3) Clean the tail warming machine's base and restraint chamber
- 4) Remove sample from ice bucket and uncap the needle
- 5) Place the animal to be injected in the tail warming machine to restrain the mouse and dilate the tail vein
- 6) Cleanse the tail with an alcohol swab
- 7) Insert the needle into one of the lateral tail veins and depress solution
- 8) Max dose 200uL
- 9) Hold an alcohol swab on site of injection for ~30 seconds, or until it stops bleeding
- 10) Dispose of needle in sharps container
 - iv. DO NOT RECAP!
- 11) Place the animal back in the cage and observe for complications

Monitoring

Watch for weight fluctuations, distended abdomen, enlarged spleens and general health deviations. Refer to your IACUC protocol for questions.

Intraperitoneal (IP) Mouse Injection

Materials

- a. 1mL syringe with 26g 3/8" needle tip containing solution of interest
 - v. Do NOT use an insulin needle even though the gauge size is correct; the length of the needle is not approved and increases the chances of improper placement and organ damage
- b. Bucket + ice
- c. Sharps container
- d. Sterile Cages
- e. Alcohol Swab
- f. RPMI (without FBS or additives)

Preparing the Needle

- 1) Prepare $10\text{-}30 \times 10^6$ strained cells/tumor in 200uL of sterile RPMI media per mouse
- 2) Draw solution into tip-less 1mL syringe
- 3) Place 26g 3/8" needle tip onto syringe and put on ice
 - vi. Be sure to line up the bevel and the numbers

Delivery of Sample

- 1) Select strain and number of mice needed
 - i. Pristane-primed NSG mice are used for intraperitoneal injections
 - ii. Non-primed NSG may also be used
- 2) Prepare a sterile cage set-up (refer to animal handling protocol)
- 3) Remove sample from ice bucket and uncap the needle
- 4) Place the animal to be injected on the food hopper and grip a large amount of skin behind the ears and above the shoulders to restrain the mouse.
 - iii. The more skin you gather in your grip, the more control you have over the animal
 - iv. A secure grip minimizes the potential for organ damage by stopping the animal from moving
- 5) Pin the tail down with your pinky on the hand that is restraining the animal and tilt the animal down to expose the abdomen
- 6) Swab the abdomen with alcohol to clean the site for injection
- 7) Insert the needle at a 30 degree angle on the animal's right side near the teat
- 8) Before injecting, aspirate the needle to make sure no urine or blood are drawn up
 - v. If you draw up blood or urine, the needle is misplaced; try again
 - vi. The left side contains the cecum while the right side is the small intestine
- 9) Depress the plunger slowly
 - vii. Max dose 200uL
- 10) Dispose of needle in sharps container
 - viii. DO NOT RECAP!
- 11) Place the animal back in the cage and observe for complications

Monitoring

Watch for weight fluctuations, distended abdomen, fluid accumulation in the abdomen and general health deviations. Refer to your IACUC protocol for questions.