

## 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 10-50e<sup>6</sup> 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 1500mm<sup>3</sup> 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.