

Adenoviral and Lentiviral Delivery to Mouse Lung

I. Prepping of Adenovirus

1. Thaw viral aliquots quickly in your hand and then place on ice prior to diluting it in EMEM.

2. Add desired i.u.'s directly into plain EMEM (Sigma Chemicals, St. Louis, MO; has the right phosphate concentration).

Notes:

A. CaPO_4 precipitates are dependent upon 1) $[\text{Ca}^{2+}]$, 2) $[\text{PO}_4]$, 3) pH, 4) temperature, and 5) time of complex formation.

B. Make sure and check the pH prior to using your stock EMEM each time.

C. Ideal Ranges: $[\text{Ca}^{2+}] =$

3. You will want 62.5 μl per mouse, so make up enough total volume for the desired # of mice + 25 μl extra volume.

BX: I use 40 μl per mouse (20 μl per nostril). This volume has been tested using trypan blue, it can distribute evenly in lung.

4. Maximum # of mice per viral precipitate = 14 (so as to ensure good precipitation in a 1.5 ml eppendorf tube).

5. Keep diluted virus on ice until immediately prior to the addition of CaCl_2 .

6. Slowly add CaCl_2 (by dripping) to a final concentration of 9.67 mM (2M stock concentration, tissue culture grade, source: Invitrogen).

7. Vortex gently to mix and incubate for 20' at RT.

8. Mix by gently pipetting up and down immediately prior to mouse delivery (use within 10').

9. You do not want the viral precipitate to sit for very long as it continues to grow in size. Too large of a precipitate impairs viral uptake/infectivity within the desired lung tissue.

Virus Delivery:

1. Immediately start intranasal virus delivery to the mice.

Note: this is as soon as the 20' Ca/PO_4 incubation (Adeno only) or any 10' pre-treatment window has been completed; coordinate timing.

2. Deliver 62.5 μl in total per mouse.

3. Each mouse receives 62.5 ul over 2 doses, delivered as follows and in the same mouse order as any pre-treatments would have been applied:

- a. 31.25 ul to nostril A, mouse 1
- b. 31.25 ul to nostril A, mouse 2
- c. 31.25 ul to nostril A, mouse 3
- d. 31.25 ul to nostril B, mouse 1
- e. 31.25 ul to nostril B, mouse 2
- f. 31.25 ul to nostril B, mouse 3

NOTES:

- Use a regular sized, filtered p200 tip for delivery.
- Change tip at every delivery to ensure equal delivery to each nostril and each mouse.

100 % Avertin Stock Solution

1 gm 222-Tribromoethanol / 1 ml tert-Amyl Alcohol (both stored in flammables cabinet)

Warm solution to 40 degrees to fully dissolve Tribromoethanol.

Store in brown bottle wrapped in foil in 4 degree.

Working Solution is 2.5% stock solution in .9% saline.

Example: .25 ml 100% stock solution in 10 ml .9% saline.

Administer .25 - .5 ml of working solution I.P. per mouse. Small mice get less, big or fat mice get more.