

Intranasal Infection Protocol

Reagents:

MEM (Sigma catalog #M4655)

2 M CaCl₂

*Adenovirus - University of Iowa

Note*:-

The University of Iowa facility sends virus at a titer of 10¹² particles/mL which is usually equal to 10¹⁰ PFU/mL. We order it aliquoted to avoid a thaw/freeze which could reduce titer. **Make sure you find out the exact PFU concentration in the lot we have in the freezer before you use it—it can vary by 2-10 fold with each batch.**

Ordering info. can be obtained from the following people:

The tech doing the ordering is:

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Protocol:

Formation of AdCre:CaP_i co-precipitates: The reaction mixture below is for an infection of one mouse using 5 x 10⁶ PFU of virus—this what we thought we were always using, but we were really using 2.5 x 10⁷ PFU. You can change the titer by altering the amount of virus used, and adjusting the volume of MEM accordingly in order to give a final volume of 125 uL. When less virus is needed, I normally dilute it in MEM and then use 2.5 uL.

Add 2.5 uL of AdCre to 121.9 uL of MEM and mix well. Next add .6 uL of CaCl₂ and mix well. Let this mixture sit for ~20 minutes prior to use.

Infection:

- 1) Anesthetize the mice with avertin, making sure that the mice are fully anesthetized and have no reaction to pain. I test this by pinching their toe with a pair of forceps. If they are not fully anesthetized they will shake their head scattering the virus.
- 2) Using a protein gel loading tip, take 62.5 uL (half) of the virus mixture and place the pipet tip at the opening of one nostril. It won't fit into the nostril, but don't that's fine. Slowly expel the virus solution from the tip. Be sure the mouse is indeed inhaling the drop that is forming. If not stop expelling and wait until it inhales what is there.
- 3) Let the mouse recover for 5-10 minutes. It will cough for a while after inhaling the virus. Wait until it's breathing has returned to normal.
- 4) Repeat with the remaining 62.5 uL of virus mixture.

Note*- Occasionally, mice don't cope with the avertin very well. It depresses their temperature and breathing rate. If you notice a mouse that is breathing really slowly or shivering I find that if you fill a large glove with hot water and place the mouse on the glove it often helps it to survive.