Crude viral lysate preparation

Infection Media: IMEM (Life tech, cat #10373-017) + 2% FBS (Hyclone Cat # SH30070-03)
All adenovirus infection and processing of infected cells should be done in BL-2 Hood under standard operating conditions required by the law. Use proper decontamination protocol.

1. Seed 5X10E5 HEK 293cells (QBI Cat # AES 0503) in 150mm plates using DMEM media (Life tech Cat #12430-054) containing 5% FBS. Incubate @ 37C until they are 70 to 80% confluent (it is important not to let them grow too much). Usually plate will be ready for infection in 2 days.

2. Dilute 1ul purified virus or 10 ul of the crude viral lysate with 2ml infection media.

3. Do not let the cells dry during the infection. Therefore aspirate the media only when everything is ready for the infection. The 293 cells are also very sensitive to the temperature always use media pre heated to 37C. Do not add the media right in the middle of the plate to avoid the detachment of cells from the plate. Add the 2-ml infection media-containing virus from step 2 gently at the corner of the plate. Incubate the plate for an hour tilting the plates every 15 min (very gently and so that the media covers all the plate) or setting a rocker in the incubator (PGC cat#75-7355-10 rock @ speed 3). After the incubation add 15 to 20ml of DMEM media containing 5 % FBS to the plates.

4. Incubate the plates until they start showing cyto-pathic effects (CPE). Typically CPE start to be evident after 24hrs and are fully developed after 36hrs. If cells have developed CPE after overnight incubation (< 24hrs) suspect another cause different from adenovirus infection (discard the preparation). We usually harvest the cells between 36 to 48 hours after infection. Detach the cells pipetting up and down with the media. Pool the recovered cells along with the supernatant in a 50ml conical tube and spin them for 10 minutes @ 2500 RPM (1000g). Resuspend the pellet in 1 to 2ml of 1X sterile PBS. Store the pellet @-20C.

5. Break open the cells by freezing (-70C, use dry ice / methanol) and thawing (37C water bath) to release the virus for 5 to 6 cycles. After each cycle vortex for 30 seconds. At the end of this step the preparation is called crude viral lysate (CVL). Spin the cell debris @ 2500RPM (1000g) for 10minutes and save the supernatant in a clean 15ml sterile tube and store @ -20C or -70C till further use.