Plaque assays of adenovirus

1. Set up appropriate cells (HeLa or 293) one day prior to use. Arrange to have cells just at confluency when used. Actual split ratio depends on cell type but rule of thumb is about 6-8 60 mm dishes per 150 mm dish of 293s, and about 20- 30 60 mm dishes for HeLa. Whenever seeding cells into 60 mm dishes for any purposes be sure to distribute evenly by slowly agitating tray before leaving it undisturbed in incubator. Be careful not to spill medium over edge.
2. Next day: prepare virus dilutions needed in PBS++.(be sure to change pipettes while making dilution series, I normally go from undiluted to 1 x 10-9). Remove medium from dishes (do not aspirate more than 10 dishes at a time or cells will dry out), Add 0.2 ml virus/dish.
3. Tilt dishes to spread virus, adsorption is fairly rapid so 30-60 mins at room temperature is adequate. Tilt once more during adsorption. If virus has been added undiluted in medium then you must incubate in a CO2 incubator ("for virus only") to maintain pH.
4. Add overlay (10ml/ 60 mm dish), which has been prepared beforehand and has equilibrated in a 440C waterbath.

   Note: Pour overlay into a 100 ml bottle for distribution and keep remaining overlay at 440C.
5. Allow overlay to harden then place dishes in appropriate VIRUS INCUBATOR.
6. On 293 cells plaques should be visible within 4-5 days and can be counted at 6-8 days. Plaques on 293 cells can be counted by eye or you can flip out overlay (by turning dish upside down !!!!) and stain monolayer of cells remaining in dish with crystal violet. Plaques on HeLa cells can be counted after staining with neutral red.

Note: For conc. of crystal violet ond neutral red communicate with Frank Grahams Lab !!, method is from his lab and we never tried staining plaques yet. Normally if you use 293 cells there is no problem to see the plaques after the 8 days period without staining cells.

Preparing the Overlay:

First prepare 400 ml 2 x F11 containing 8 ml pen/strep., 8 ml L-glutamine, 8 ml 5% yeast extract (=F11+++). This can be stored a few weeks at 40C.

For 200 ml complete overlay (20 dishes) mix together:

- 90 ml 2 x F11 +pen strep, +L-gln, + yeast ext.
- 10 ml heat inactivated HS
- 100ml of 1% agarose (MQ water + 1g agarose).

Note :1% agarose has to be autoclaved and both the 2 x F11mix and 1% agarose have to be adjusted to 440C before mixing. After mixing use within about one hour.

In general:

For the overlay mix the media, which is used for the virus infection assay is always the most appropriate to use. Therefore for 293 cells use 2 x F11 or 2x à-MEM.

5% yeast extract: mix with MQ water and autoclave.

2 x F11: purchase from cancer research group

2x à-MEM: You have to make this yourself

L-glutamine: purchase from GIBCO/BRL stock conc. 200 mM

PBS++: add 1 ml each of 1 and 2 to 100 ml PBS. Virus is supposed to bind to and infect cells more efficiently.

1. 100 x stock $CaCl_2 \times H_2O$ (1g/100 ml)

2. 100 x stock $MgCl_2 \times 6\ H_2O$ (1g/100 ml)