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Use of P4.CCR5 cells for quantitating HIV infectivity

P4.CCR5 cells are HeLa cells that contain a transfected LTR-β-gal construct, CD4 expression vector and CCR5 expression vector. They are similar to Mike Emerman's MAGI cells and can be used similarly (Kimpton and Emerman, (1992) J. Virol. 66:2232) They differ in that they express CCR5 and can, as a result, be infected by M-tropic virus isolates. The cells are based on P4 cells of P. Charneau (Clavel and Charneau, J. Virol. (1994) 68:1179) which he derived by screening through a large number of HeLa LTR-β-gal transfectants to find one that could be transactivated but had a negligible number of blue cells in the absence of virus.

To ensure that CCR5 expression is maintained, the cells should be cultured in DMEM/10% FBS containing 0.5 μg/ml puromycin. Drug selection for CD4 does not seem to be required.

Typical protocol for use of P4.CCR5 cells.

1. The day before infection, plate cells in 6-well dishes at 1×10^4/well in medium without drugs.

2. The next day, remove medium. Add virus (1-100 ng p24^{gag} in a volume of 0.5 mls. Polybrene (4 μg/ml) can be added to boost titer if desired).

3. Remove virus and replace with fresh culture medium (no drugs).

4. Two to three days later, stain the cells for β-gal in situ. Alternatively, β-gal can also be measured by lysing the cells. Count blue cells in a defined area under a dissecting microscope.

Notes:

1. The cells replicate most T and M-tropic viruses; however, one person said that many primary isolates would not replicate on these cells. Thus, it is important to confirm that the virus used does replicate in these cells.

2. Luminescent detection of β-gal is very sensitive, rapid and reliable and would be a good way to quantitate infection of the cells. It does not provide the number of infected cells, but can be used comparatively for evaluating neutralizing antibodies or HIV inhibitors. The Tropix reagent works well for producing long lived light emission.

3. β-gal can also be detected by FACS-GAL, but we have not tried this.