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**MAGI-CCR5 ASSAY**

We have developed an indicator cell line (MAGI-CCR-5 cells) that allows detection of both macrophage tropic and T-cell adapted strains of HIV after only a single cycle of replication. The MAGI-CCR-5 cells are sensitive to all isolates of HIV we tested. Furthermore, MAGI-CCR-5 cells are also susceptible to SIV, including: SIVmneCL8, SIVmac239, SIVmnel70, and SIV 1A11. This cell line is described in: Chackerian B, Long EM, Overbaugh J. HIV-1 co-receptors participate in post-entry stages of the virus replication cycle and function in SIV infection. *J. Virology* **71**:3932, 1997.

**ABOUT THE CELLS** - The parental cell line, MAGI, was developed by M. Emerman's laboratory (Kimpton J, Emerman M. *J Virol* **66**:2232, 1992). We have constructed these cells to express the β-chemokine receptor, CCR-5 (also known as CCR-5), by infecting MAGI cells with the amphotropic retroviral vector MULV (PA317) carrying the CCR-5 construct developed in Dan Littman's lab (Deng et al. *Nature* **381**:661, 1996). A single clone was selected and tested for functional of CCR-5 expression by using macrophage tropic virus, SF162.

**CARE OF MAGI-CCR5 CELLS** - Cells are grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U per ml penicillin, 100 µg per ml streptomycin, 0.25 µg/ml fungizone, and 300 µg per ml glutamine (complete DMEM). A week after thawing an aliquot of cells, passage the cells in this medium plus 0.2 mg/ml G418 (at an active concentration of 700 µg per mg), 50 U/ml hygromycin (from CalBiochem-with a concentration of 358 Units/mg), and 1 µg/ml puromycin. We only add G418, hygromycin, and puromycin when passaging the MAGI-CCR5 cells. We do not use these drugs during the week after thawing or during the assay.

Thawing cells from liquid nitrogen - There are approximately 5 x $10^6$ cells per vial. Thaw cells and plate directly into a T-25 flask in about 5 ml of medium (see above). Replace the medium once the cells adhere to remove the residual DMSO. We recommend using medium without G418/hygromycin/puromycin during the first 5 to 7 days after thawing. Passage the cells for a week or so before attempting the assay, taking care to establish the cells in drug selection media.

Typically, we trypsinize and split the cells 1–2 times a week at dilutions of 1/10 to 1/20 when they reach confluency.

Freeze aliquots of the cells as soon as possible. The developers of the MAGI cells found that the longer the cells are passaged the less CD4 they will express, leading to reduced effectiveness of the assay. As a general rule, we thaw a new aliquot of MAGI-CCR-5 cells every 2–3 months (about every 20th passage). If necessary, you can screen/select for CD4 expression by FACS (Kimpton and Emerman, *J Virol* **66**:2232, 1992). Freeze the cells in DMEM with 20% fetal bovine serum, 10% DMSO. On average, a confluent T-75 flask yields 10 vials of frozen cells. We have found that after approximately 8 passages, the cells change morphology by becoming balloon shaped and begin to clump after trypsinization. This does not appear to affect the assay. We have determined that after 3–4 months in culture (18–24 passages), the viral infectivity remains essentially constant compared with that of freshly thawed cells.

**THE ASSAY** - Typically we infect the MAGI-CCR-5 cells in either 12 or 24 well plates. In some cases, we've found that the 24 well plates yield slightly lower numbers of infected (blue) cells, presumably because of the reduced number of target cells, but they are considerably less labor intensive to count.

(DAY 0) MAGI-CCR-5 indicator cells are plated in 12-well plates at 8 x $10^4$ cells per well (4 x $10^4$ cells per well for a 24-well plate) in complete DMEM plus G418/hygromycin/puromycin the day before infection. The cells should be 30–40% confluent the day of infection.

(DAY 1) The cells are infected by removing the medium from each well and adding dilutions of virus in a total volume of 300 µl of complete DMEM (without G418/hygromycin) with 20 µg per ml DEAE-Dextran (150 µl for 24-well plate). The cells are extremely sensitive to DEAE-dextran and you should not exceed 20 µg/ml. After 2 hours incubation (at 37°C in a 5% $CO_2$ incubator) add 1.5 ml complete DMEM (1 ml for 24 well plate) to each well. Incubate cells for 2 days at 37°C in a 5% $CO_2$ incubator.

(DAY 3) Remove media and add 1-2 ml fixing solution per well. (Fixing solution is: 1% formaldehyde, 0.2% glutaraldehyde in PBS. It can be made up in advance and stored indefinitely at ~~4~~0°C away from light. For 100 ml, use 2.7 ml 37% formaldehyde, 0.8 ml 25% glutaraldehyde). Fix for 5 minutes (and no longer). Remove fix and wash cells twice with PBS.

Add staining solution to cells, 600 µl (12 well plate) or 400 µl (24 well plate) per well. Incubate the plates for 50 minutes at 37°C in a non-$CO_2$ incubator. Staining for over 50 minutes can cause background problems. The staining is stopped by removing the staining solution and washing thoroughly at least two times with PBS. Some care must be taken here; while the cells are adherent they have been known to come off the plates on occasion.

Staining solution is: (for each ml)

949 µl PBS

20 µl 0.2M potassium ferrocyanide

20 µl 0.2M potassium ferricyanide

1 µl 2M $Mg_2Cl$

10 µl 40 mg/ml X-gal (make up X-gal in DMSO and store at -20°C in small aliquots that are protected from light with aluminum foil)

**Troubleshooting - Some common problems**

Cells look dead or are sparse in the middle of the well?

Probably the DEAE-Dextran concentration was too high. Repeat with lower concentration of DEAE-Dextran. Alternatively, the cells may be drying out after removal of medium and before adding dilutions of fresh virus. Remove medium from six wells at a time, apply virus dilutions, and move onto the next set of six wells.

High Background?

1) Your cells may have been in culture too long. Typically, we thaw out a new vial of cells every 2–3 months, although we have successfully used MAGI-CCR-5 cells in this assay after 3+ months in culture.

2) Alternatively, you may have stained for too long. For best results, stain for 50 minutes.