Culture and Syncytium Detection Using CHO-Env Cell Lines

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The transfected CHO cells described in this protocol express wild type (CHO-WT), secreted (CHO-SEC), or GPI-anchored (CHO-PI) HIV-1 envelope glycoproteins, and a control cell line (CHO-EE) that lacks HIV sequences. The protocol outlines the culture conditions and provides a simple assay for cell fusion and fusion inhibition studies.

Reagents:	CHO Cell Derivatives CHO-SEC (Catalog #2237) CHO-EE (Catalog #2238). CHO-WT (Catalog #2239) and CHO-PI (Catalog #2284) will be available for release during the summer of 1994.
$CD4^+$ Cells	HeLa $T4^+$ (Catalog #154)
GMEM-S Culture Medium	Glutamine-deficient minimal essential medium (see attached formulation)
Selection Medium	GMEM-S containing 400 µM methionine sulfoximine (MSX)
Cell Detachment Medium	PBS containing 0.5 mM EDTA and 0.5 mM EGTA
Freeze Medium	Propagation medium 60%; FBS 30%; DMSO 10%; no MSX

Cell Culture Procedure:

1. Rapidly thaw the cells by warming the vial in a 37°C water bath. Immediately upon thawing, add the cells to 10 ml of GMEM-S.

2. Centrifuge the cells at 800 rpm for 5 minutes. Resuspend the pellet in 10 ml of GMEM-S and transfer to a 10 cm dish or a T25 flask. Incubate the cells overnight at 37°C in a 5% CO_2 humidified atmosphere.

3. The next day, aspirate the supernatant and replace it with selection medium.

4. After 24-48 hours, the cells should be near confluency. At this time, split the cells at a 1:10 ratio and continue to propagate in 10 cm dishes or transfer into larger T-flasks.

5. Continue to maintain the cells in selection medium, splitting about 1:12 every 3-4 days such that they reach confluency in 3-4 days. These cells lose viability if confluent for more than 1 day.

6. To freeze the cells, resuspend at a concentration of 3-5x10^6 cells/ml in cold freeze medium. Retain low passage stocks of the cells in liquid nitrogen, as the envelope expression will drop after repeated passages. Envelope expression can be transiently increased (at least two-fold) if sodium butyrate is added 15-18 hours before the cells are harvested. A range of 3-12 mM sodium butyrate is optimal, but should be independently determined for each of the different cell types.

Syncytium Assay:

1. Culture the HeLa $T4^+$ cells and CHO-WT cells in 10 cm dishes to near confluency.

2. Rinse the cells one time using the cell detachment medium, then add 2 ml of fresh detachment medium. Incubate the dish at 37°C until the cells detach. Gently tapping the dish a few times may facilitate the detachment.

3. Coplate 0.2 ml of each cell type in a 6-well dish; each well should already contain 3 ml of GMEM-S. Incubate the cells overnight at 37°C in a 5% CO_2 humidified atmosphere.

4. The next day, view the syncytia under the microscope. The cultures can be photographed at this point.

5. To fix the cells, aspirate the supernatants and rinse the cells one time with PBS, then add 1 ml of 10% formaldehyde-PBS for 3 minutes at room temperature. Wash the cells one time with PBS, aspirate the solution and stain the cells with 1 ml of Giemsa dye.

GMEM-S Propagation Medium for CHO-Env Cells:

To make 1 liter:

704 ml sterile water
100 ml 10X MEM w/o L-glutamine (Gibco Cat. #11430-014)
100 ml FBS (can use supplemented bovine calf serum)
36 ml 7.5% sodium bicarbonate (Gibco Cat. #25080-011)
20 ml 50X nucleosides (Sigma - see below)
10 ml 100X glutamate + asparagine (Sigma - see below)
10 ml 100 mM sodium pyruvate (Gibco Cat. #11840-030)
10 ml penicillin-streptomycin 5000 U/ml (Gibco Cat. #15070-014)
10 ml non-essential amino acids (Gibco Cat. #11140-019)

Sigma reagents for 50X nucleoside mix:
35 mg adenosine (Cat. #A4036)
35 mg guanosine (Cat. #G6264)
35 mg cytidine (Cat. #C4654)
35 mg uridine (Cat. #U3003)
12 mg thymidine (Cat. #T1895)

Make in 100 ml ddH_2O and filter-sterilize through a 2 µ filter. Store frozen.

Sigma reagents for 100X G+A:
600 mg L-glutamic acid (Cat. #5638)
600 mg L-asparagine (Cat. #A4159)

Make in 100 ml ddH_2O and filter-sterilize through a 2 µ filter. Store frozen.

For complete selection medium, supplement with 400 µM methionine sulfoximine (MSX) (Sigma, Cat. #M5379). Prepare MSX stock at 18 mg/ml in medium, filter-sterilize, and store at -20°C.