HeLa SPINNER CULTURES induced by Interferon alpha and gamma:

Note: Protocol is identical to HeLa S3 protocol, except for portions highlighted in red.

From: Duke/UNC/UT/EBI ENCODE group
Date: 11/30/09
Prepared by: Crawford lab

Use Hela S3 cells which have been adapted for growth in spinner culture.
For ENCODE, the ATCC number for HeLa-S3 is CCL-2.2 and the lot number is 4490244.

Interferon

Recombinant human interferon alpha from PBL inteferonsource
catalog #11100-1
lot # 4153

Recombinant human interferon gamma from R&D systems
catalog #285-IF

Procedure:

1. Frozen cells should be thawed into a 150 mm dish containing DMEM+5% calf Serum+1% P/S; incubate @37°C, 5% CO2 until cells reach about 60% confluency. There will be about 1-2 x 10^7 cells on this dish.

2. Trypsinize cells, resuspend in **Initial Spinner Medium**, and count.

Initial Spinner Medium: S-MEM Joklik's+ Alpha MEM (1:1ratio) + 5% calf serum+1% P/S

3. Add **Initial Spinner Medium** to the trypsinized cells so that cells are at a density of 1-2x10^5 cells/ ml and transfer to 500ml spinner flask containing 200 ml medium (this results in a spinner culture of cells at an initial density of 1-2 x 10^5/ml. Spin at slow-medium setting (in our case is #2), sufficient to maintain good aeration, but slow enough to avoid damaging the cells.

4. Expand cells when they reach 5 x 10^5 cells/ml by transferring into larger spinner flasks, using **Expansion Spinner Medium**. When expanding, dilute to 1-2 x 10^5/ml and harvest when cells reach 5 x 10^5 cells/ml.

Expansion Spinner Medium: S-MEM Joklik's+ Alpha MEM (4:1) + 5% calf serum+1% P/S

5. Cells were induced by 5ng/mL IFN-gamma and 500U/mL IFN-alpha, respectively for four hours.

Comments:

1. We thaw the cells onto a dish, instead of directly into spinner culture, because we have found that the survival is much higher using this method.

2. The Initial Spinner Medium has a higher ratio of Alpha MEM than the Expansion Spinner Medium because the higher calcium in the Alpha MEM is helpful when initially establishing the cells in spinner, but can lead to problems with clumping in the larger flasks.

3. Spinner flasks should not be filled more than half way to maintain sufficient aeration; as cell number increase, larger sized flasks will have to be used. Side arm caps must be kept loose to allow for air exchange. Check carefully for contamination - it is often hard to distinguish this from cell debris, which accumulates as cells are maintained in spinner culture. After use, treat the emptied spinner flasks with a 10% bleach solution for 30 minutes and then thoroughly rinse with distilled water. Add a small amount of dH2O to flasks before autoclaving to avoid breakage during autoclaving - decant prior to use.