Systems Virology
of Emerging Respiratory Viruses

TCL006.0P - Treatment of Calu-3 cells with Interferon α

(4-12-10, Lynn Law)

This protocol is designed for cell culture using 6-well plates and 2 ml of medium/well.

I. Preparing Interferon α working solution (Sigma I4276)

Vial contains 20μg in buffered solution.

Using sterile technique:
To make a 200X solution (2 μg/ml):

1. Bring INFα up to 10 mls of buffer which contains at least 0.1 % BSA. Aliquot into microfuge tubes or vials. Label with contents, date, initial and anything else you think is necessary. Aliquots should be stored in refrigerator.
2. Make 1 X working solution just prior to use - Assuming final concentration of 10 ng/ml and 2 ml/well. Add 200ul of 200X stock solution into 39.8 ml of cell culture medium.

II. Treating cells with Interferon α

Set up:

- Label tubes with permanent ink.
- Make Interferon α working solution (2ml/well)
- Cell culture medium
- Cold 1 X PBS
- Dry ice
- TRIZOL Reagent

1. Remove medium from wells.
2. Add 2 ml of 1X IFNα cell culture medium to each well to be treated.
3. To the time matched mock well add 2 ml of cell culture medium without IFNα.
4. Start timing once treatment is added to the cells.

III. Collecting samples for RNA analysis

- Samples are collected following protocol TCL001.0P

1. Harvest T=0 samples just before proceeding with Step II above.
2. Harvest samples post-treatment according to experimental design.