EXPERIMENT:
Test of IkBaM adenovirus on cell growth in HeLa cells.

REAGENTS

Cells: HeLa cells.

Virus: IkBaM Adenovirus or control adenovirus in PBS containing 3% Sucrose.

MTT solution: 3-(4, 5 dimethylthiazol-2-yl)-2, 5 -dimethyltetrazolium bromide) (MTT) A 0.5mg/ml solution was prepared in PBS.

SDS/DMF reagent: (20% SDS in 50% DMF): A 40 % solution of SDS was first prepared in water. This solution was diluted by half with N,N'Dimethylformamide.

PROCEDURE

This protocol is written for HeLa cells. However, this can be adapted to the cell line of your choice.

Preparation of cells:
1. Prepare HeLa cells grown to confluence in a 100 mm culture dish.
2. Trypsinize, wash the cells and seed at 10,000 cells per well in a 96-well plate.
3. Twenty-four hours later replace the medium with DMEM containing 2% fetal bovine serum.
4. Infect cells with the control or test virus at various dilutions.

Recommended concentration is 1:100, 1:1,000 and 1:10,000. Dilute the viruses in DMEM containing 2% fetal bovine serum (1:100 dilution would result in approximately 100 viral particles per cell).

MTTAssay:
1. After 24 hrs, remove the medium and wash the cells with PBS.
2. Add twenty-five micro liters of MTT solution per well. After 40 minutes of incubation at room temperature, add 100 ul of SDS reagent was added per well.
3. Read the plate in a micro-plate reader using the filter 595 nm filter.