Protocol for HeLa cell culture and plasmid transfection

AV-02 HeLa cell culture

1.0 Introduction / Description

This method is intended for maintenance of Tet-ON HeLa cells.

2.0 Materials

Glassware/Plasticware

10cm cell culture dishes- CORNING cat.# 430167

Reagents

1X pen/strep (Penicillin/Streptomycin) GIBCO cat.# 15140
DMEM 1X (Dulbecco modified Eagle medium) GIBCO cat.# 11965
Tet system approved FBS Clontech cat. #631106
PBS 1X (Phosphate buffered saline pH7.4) GIBCO cat.# 10010
Tryple 1X GIBCO cat.# 12604
Tet-ON HeLa cells- Clontech HeLa Tet-On® 3G Cell Line cat.# 631183

3.0 HeLa cell culture

Tet-ON HeLa cells are cultured using DMEM media (Gibco) supplemented with 10% Tet system approved FBS (Clonothec) and 1X pen/strep (100 units of penicillin, 100 μg of streptomycin; Gibco).

- ✓ Cells are maintained in 10cm dishes at 37°C and 4% CO_2 until 60-80% confluency
- ✓ When confluent, cells are split 1:10 – 1:20 with the following procedure:
    - Wash cells once in PBS buffer (Gibco)
    - Add 2ml of Tryple solution (Gibco) to the cells
    - Incubate cell for 2 minutes at 37°C or until completely detached from the plate
    - Add 5 ml of complete media to the cell/Tryple solution
    - Replate cells at a 1:10 - 1:20 dilution in new 10 cm plates.

AV-03 Transfection of the HuEV-A constructs in HeLa cells

1.0 Introduction / Description

This method is intended for the transfection of the previously subcloned HuEV-A constructs into Tet-ON HeLa cells. The transfection is performed in 6 well plates using a lipid-based transfection (Fugene-HD). Each plate is dedicated to the production of cell lysates to be used for a single antibody validation. This setting provides sufficient amount of proteins for immunoprecipitation with three different antibodies (control normal mouse IgG, antibody of interest and anti FLAG-M2 antibody).

2.0 Materials

Glassware/Plasticware

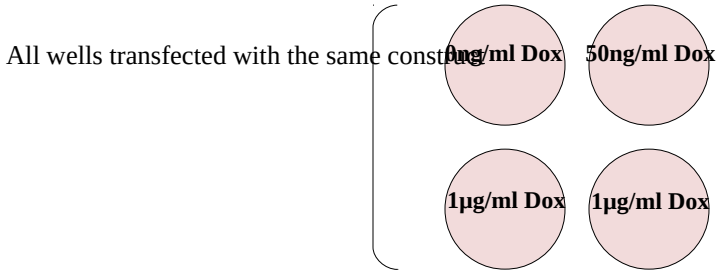
100mm TC-Treated Culture Dish-Corning cat#430167
5 ml polypropylene tubes- Eppendorf cat.# 0030119487

Reagents

Fugene-HD- Promega cat.# E2311
Opti-MEM (Reduced serum medium)- GIBCO cat.# 31985
DMEM 1X (Dulbecco modified Eagle medium)- GIBCO cat.# 11965
Tet system approved FBS Clontech cat. #631106
Tet-ON HeLa cells- Clontech HeLa Tet-On® 3G Cell Line cat.# 631183

3.0 Transfection

- ✓ Seed 2x10^6 Tet-ON HeLa cells per 10cm^2 plates with 10 ml of complete DMEM media per well. Each construct encoding a specific protein will be transfected in a set of four 10cm^2 plates in order to treat cells with 3 concentrations of doxycycline (0, 50 or 1000 ng/ml). The 50 ng/ml and 1000 ng/ml doxycycline treatments are performed in duplicate and triplicate respectively to obtain enough lysate for the IP assay:



- ✓ Incubate cells over night at 37°C and 4% CO2
- ✓ For each well:
  - Mix 24 µl of Fugene-HD solution with 600µl of OPTIMEM and add this Fugene/OPTIMEM mix to 8µg of plasmid generated in section 1
  - Incubate at room temperature (RT) for 20 minutes
  - Add the transfection mixture drop-wise to the cells.
- ✓ Incubate at 37°C and 4% CO2 for 48-72 hours.