Cell culture

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Materials: *Reagents:* High glucose (HG; 4.5g/L) Dulbecco's modified Eagle's medium containing L-glutamine (580 mg/L) and sodium pyruvate (110 mg/L) (DMEM; Gibco); low glucose (LG; 1g/L) DMEM (Gibco); heat-inactivated fetal bovine serum (FBS; Gibco); antibiotic antimycotic solution (PenStrep; 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.25 µg/mL amphotericin B; Sigma-Aldrich); 0.5% Trypsin-EDTA (Gibco); sterile calcium/magnesium free phosphate buffered saline (PBS, Sigma-Aldrich); 50 mM Tris-HCl buffer (pH 7.0)

Equipment: Cell Incubator (37 °C, 5% CO2, humidified), Cell culture plastics e.g.dishes/ flaskes/ multiwell plates

Procedure: Human cancer cell lines were grown as monolayer cultures in HG DMEM supplemented with 10% (v/v) FBS and 1% (v/v) PenStrep. Cultures were incubated at 37°C in a humidified atmosphere with 5% CO_2, and the medium was refreshed every second day. To maintain cultures in the active logarithmic phase of growth, cells were passaged once a confluency of 70 – 80% was reached. To passage the cells the medium was removed and the cells were incubated with 0.05% (w/v) Trypsin-EDTA in sterile PBS for 5 – 10 min. The trypsin was then quenched by addition of twice the corresponding volume of DMEM supplemented with 10% (v/v) FBS and the cells were centrifuged at 100 × g for 3 min at room temperature. After discarding the supernatant, the cell pellet was resuspended in desired volume of medium and cells were equally split into new tissue-culture plates/flaskes to continue cultering.