Cell cultures

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Mammalian cell lines

HEK293 is a human cell line derived from kidney cells and grows in a monolayer culture. Cells were grown in DMEM medium supplemented with 10% heat-inactivated FBS.

HEK293T cell line is derived from HEK293 cells. HEK293T cells express the SV40 large T-antigen that enables episomal replication of plasmids containing the SV40 origin of replication in transfected cells. Cells were grown in DMEM medium supplemented with 10% heat-inactivated FBS.

Subculturing monolayer cell cultures

- Culture medium from a T-75 flask containing a monolayer of HEK293, HEK293T was removed and discarded
- T-75 flask was rinsed with 10 ml of PBS buffer to remove all traces of growth medium which inhibits trypsin activity.
- 2 mL of trypsine solution was added and the flask was gently tilted to ensure the trypsin solution covered all cells. Cells were incubated in trypsin for 0.5 - 2 minutes.
- When the cells started to detach from the surface, 8 mL of growth medium was added to the trypsin solution. All remaining cells were resuspended from the bottom of the T-75 flask by pipetting.
- Transfer the cell suspension was transferred to a 15 mL centrifuge tube and centrifuged for 5 min at 1200 rpm.
- Trypsin-containing medium was removed from the centrifuge tube and cell pellet was resuspended in fresh medium.

Cell plating

- Number of cells was determined on Countess Automated Cell Counter (Invitrogen).
- The desired number of cells per well was calculated.
- Cells were diluted in DMEM with 10% FBS, transferred to an appropriate plate and placed in a cell culture incubator.

Transfection

- Dilute plasmid DNA to was diluted to desired concentration in 150 mM NaCl, gently vortexed and briefly spinned down.
- Transfection reagent PEI was diluted in 150 mM NaCl, gently vortexed and briefly spinned down.

- PEI solution was added to the DNA solution.
- The transfection mix was vortexed immediately, briefly spinned down and incubated for 20-30 min at room temperature.
- The transfection mix was added to the cells by gentle pippeting.

Preparation of transfection mixes for different cell culture formats:

Culture format	PEI reagent per µg of DNA (µL)	Amount of DNA (ng)	Volume of 150 mM NaCl and PEI (µL)	Total transfection mixture volume (µL)
96-well	12	200	10	20
24-well	12	500	50	100
8-well microscope slide	12	500	50	100
12-well	12	1000	50	100
6-well	12	2000	100	200
10 cm petri dish	12	15000	250	500

Media and buffers

DMEM supplemented with: 1% L-Glutamine (GlutaMax), 10% FBS.

Viability test

For the determination of the toxicity of gas vesicles/proteases, viability of the cells was measured with an automated cell counter (Countess, Invitrogen).

- HEK293T cells were seeded at 105 cells per well in a 24-well plate and upon reaching 60-70% confluency transfected with PEI. Cells were transfected with plasmids encoding for gas vesicle-forming proteins (GvpA, GvpC) or proteases TEVp, TEVpE and TEVpH.
- 24 h after transfection cells were gently removed from the surface of the well by pipetting and transferred to an Eppendorf tube.
- 10 µL of cells was mixed with 10 µL of trypan blue dye and viability of the cells was determined on the cell counter.