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We use this protocol and it's working

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Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

ASAP Collaborative Research Network

Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

ABSTRACT

This protocol describes routine cell culture maintenance.

MATERIALS

Reagents

- Dulbecco Modified Eagle Medium (DMEM, Thermo Fisher)
- Fetal Bovine Serum (FBS, Thermo Fisher)
- HEPES (Thermo Fisher)
- non-essential amino acids (NEAA, Thermo Fisher)
- Penicillin-Streptomycin (Thermo Fisher)
- Iscove's Modified Dulbecco's Medium (IMDM, Thermo Fisher)

Cell culture

5m

1 Grow HeLa and HEK293T cells in Dulbecco Modified Eagle Medium (DMEM, Thermo Fisher) supplemented with 10% (v/v) Fetal Bovine Serum (FBS, Thermo Fisher), [M] 25 millimolar (mM) HEPES (Thermo Fisher), 1% (v/v) non-essenMal amino acids (NEAA, Thermo Fisher), and 1% (v/v) Penicillin-Streptomycin (Thermo Fisher).

2 Culture HAP1 cells in Iscove's Modified Dulbecco's Medium (IMDM, Thermo Fisher), supplemented with 10% (v/v) Fetal Bovine Serum

(FBS, Thermo Fisher) and 1% (v/v) Penicillin-Streptomycin (Thermo Fisher).

3 Regularly test all cell lines for mycoplasma contaminations.

4 Split the cells by trypsinization once confluent.

5 To this end, remove medium with Pasteur pipette.

6 Wash cells with 1x PBS and then add trypsin for 00:05:00 at 37 °C. 5m

7 Afterwards, resuspend cells in medium and retain 10% for further culturing and expansion.

8 Discard the rest or collect for seeding for experiments.