Version 2, February 5th 2018 by Sebastiaan Bol, approved by Dr. Bunnik

Changes with respect to version 1:

- Now 5% FBS, no longer 10%
- No more antibiotics added
- DMEM only added to TrypLE instead of medium containing FBS (centrifugation step)

1. Background information

293T cells are from human embryonic kidney origin. They are epithelial, adherent cells.

Cells should be cultured at biosafety level 2 because it contains an adenovirus.

Cell line (product no. CRL-3216) was purchased from ATCC (https://www.atcc.org/Products/All/CRL-3216.aspx)

Cells should be stored in the vapor phase of liquid nitrogen.

293T cells are highly transfectable cells that contain the SV40 T-antigen.

2. Culture medium

DMEM *1 (Gibco 11995065 or ATCC 30-2002)	500 ml
Contains:	
4 mM L-Glutamine, 4.5 g/L glucose and 1 mM sodium pyruvate	
Add:	
Heat inactivated **FBS** *2 (**5**% f/c)	25 ml
L-glutamine *2 (Corning 25005CI, 200 mM) (final total concentration 6 mM)	5 ml

*1, stored in the fridge

* 2, stored as aliquots in the -20C freezer

3. CULTURING / PASSAGING

WORK STERILE. EVERYTHING GOING INTO THE FLOW CABINET NEEDS TO BE WIPED OFF WITH A TISSUE WITH 70% ETHANOL. THIS INCLUDES YOUR HANDS/GLOVES!

WHEN CELLS ARE TESTED FOR MYCOPLASMA (BI-WEEKLY) AND THE RESULTS WERE NEGATIVE, YOU MUST WORK IN THE MYCOPLASMA NEGATIVE FLOW (but only if you have obtained your culture diploma; cell that have been cultured in the left flow cabinet (where potential mycoplasma material is handled) cannot (never) be transferred to the mycoplasma negative flow cabinet).

Look at the cells under the microscope and determine confluence and general appearance (morphology). Write both down in your lab journal.

Numbers are for 25 cm^2 culture flasks. See table below for 75, 175 and 225 cm^2 flasks.

- Warm PBS and medium to 37°C using the bead bath.
- Remove old medium (5 ml) with pipet and discard into liquid waste bottle inside the flow cabinet.
- Add 5 ml pre-warmed PBS to the flask (not directly onto the cells) and rinse briefly by swirling. Discard PBS.
- Add 1.25 ml room temperature TrypLE to the flask. Swirl gently and incubate 2-3 min. Do not shake or hit the flask as this will increase the formation of clumps of cells. Observe under microscope if cells detached. When you see that most (about 75%) of the cells are detached, you can continue. When you see all cells detached, you waited too long.
- Add 2.5 ml (double the amount of TrypLE used) pre-warmed **DMEM only** and dispense over the cell layer surface several times.
- Transfer to 15 ml tube and centrifuge at 130 x g for 5 min and discard sup.
- Resuspend cells (careful, these cells are believed to be sensitive to bubble bursting) in 2 ml pre-warmed medium, take sample for cell count (at least 15 µl) and count the cells. Make sure the cells are distributed evenly across the medium. Clumps are not acceptable, because this will give a highly imprecise cell count.
- Take a fraction of the 2 ml containing cells and add pre-warmed medium to end up with **15,000 living cells / cm^2 (375,000 cells total) on Monday and Wednesday (48 hours) OR 6,000 cells / cm^2 (150,000 cells total) on Friday (72 hours), in a total of 5 ml medium**.

- Passage every **Monday** (after 72 hours), **Wednesday** (after 48 hours) and **Friday** (after 48 hours) to remove old medium with waste, add fresh medium with nutrients, and to split cells to prevent overgrowing.

- Label each flask with the date, your initials, cell type name and passage no.

- Write the following in your lab journal: confluence, cell appearance, cell viability (when dead/living cell count with trypan blue was done), cell number counted etc.

- Place cells (back) inside the 5% CO_2 / 37°C incubator.

- Inside the incubator, briefly and gently swirl medium in the flask while it is in horizontal position for equal distribution of the cells over the culture flask surface.

	25 cm^2	**75 cm^2**	**175 cm^2**	**225 cm^2**
Medium (flask horizontal)	5 ml	15 ml	35 ml	45 ml
PBS [1]	5 ml	15 ml	35 ml	45 ml
TrypLE [2]	1.25 ml	3.75 ml	8.75 ml	11.25 ml
Medium to be added before centrifugation [3]	2.5 ml	7.5 ml	17.5 ml	22.5 ml

[1] 2 ml per 10 cm^2

[2] 0.5 ml per 10 cm^2

[3] Double the amount of TrypLE used

Cells can reach max. 80% confluence. After this cells will no longer be in the log phase and will die off.

Maximum passage number 30 – 50 ×.