# 1. Cell Cultivation

*NB 1: Always use plastic gloves and work in the sterile environment inside a LAF cabinet. Clean the cabinet with 70 % ethanol. Spray gloves, working surfaces, microscopes, centrifuges, incubators etc. with 70 % ethanol if you suspect contamination.*

*NB 2: Cell suspensions are to be thoroughly mixed before any transfer where a correct cell concentration is of importance.*

*NB 3: Always pre-heat media before adding cell suspensions for cultivation.*

## 1.1 Subcultivation

*Materials:*

- 1×PBS + EDTA
- Trypsin (1×)
- cell growth media

*NB: The volumes below are for 10 cm Petri dishes. Double the volumes when using 15 cm dishes.*

1. Remove the medium from the Petri dish using a stripette or the VacuBoy.
2. Add 10 ml PBS + EDTA.
3. Remove the PBS + EDTA after ~ 5 minutes.
4. Add 1 ml Trypsin (1x).
5. Incubate in 37 ˚C and 5.2 % $CO_2$. for 3 min (+ 1 minute if needed).
6. Inspect the Petri dish to ensure that the cells are detached.
7. Add 1 ml of medium to the dish and separate the cells by pipetting (1 ml pipette).
8. Add 10 ml cell growth medium to a new Petri dish.
9. Add the detached cells to the new Petri dish.
   1:5 400 µl cell suspension
   1:7.5 267 µl cell suspension
   1:10 200 µl cell suspension
   *NB: If the cells are to be split less than 1:5, the trypsin needs to be removed. Pellet the cells by centrifugation (1700 rpm, 3 min), remove the supernatant and reconstitute the pellet in 1 ml fresh growth media.*
10. Swirl the Petri dish gently and inspect it in the light microscope.
11. Cultivate in humified air at 37 °C (5.2 % $CO_2$ ).

## 1.2 Thawing Cell Batches

1. Pre-heat a 50 ml Falcon tube with Staq water by immersing it in warm water (screw on lid tightly).
2. Pre-heat a 10 cm Petri dish, marked with the date, your name and the passage number, containing 10 ml of culturing media in the incubator.

3. Retrieve the cells from the – 80 °C freezer.
   *NB: Keep the vials on dry ice when retrieving multiple samples. If dry ice is not available, only retrieve one vial at a time.*
4. Place the vial in the Staq filled Falcon tube until the cells are about half thawed.
5. Add a few drops of the pre-heated media to the tube, to avoid osmotically shocking the cells. Carefully homogenize the solution by pipetting.
6. Transfer the cell suspension to the Petri dish and inspect the cells in the micro-scope.
7. Cultivate in humified air at 37 °C (5.2 % $CO_2$ ) in the upper incubator.
8. Filter and re-add 5 ml of the media the next day and replenish with 5 ml of new media.

## 1.3 Freezing Cell Batches

*NB: At least 1 500 000 cells, but preferably 2 000 000 – 2 500 000 should be frozen in each cryo tube.*

1. Detach, centrifuge and resuspend the cells in 1 ml of medium.
2. Count the cells to determine the cell concentration.
3. Centrifuge the cell suspension and resuspend the pellet in a desired multiple of 100 µl cell cultivation media. The cell suspension is to contain 1.5 – 2.5 million cells per 100 µl portion after resuspension.
4. Prepare an equal amount of cryo tubes containing 800 µl (20 % FBS) and 100 µl filtered DMSO (10 %). Mix thoroughly and label all tubes with date, cell line, pas-sage number and your initials.
5. Add 100 µl of cell suspension to each cryo tube and mix the solution thoroughly.
6. Put the vials in a CoolCell-container and immediately place it in the – 80 °C freezer (The cells will not survive over night in the – 20 °C freezer.).
7. Move the cells from the CoolCell to the designated storage box the next morning.

## 1.4 Liquid Nitrogen Storage

*NB: Only handle liquid nitrogen tanks in well ventilated areas. While refilling the nitrogen tank, keep the gas room door open. Since $N_2$ expands volumetrically 700 times when evaporating, there are suffocation hazards. Always wear the protective clothing and equipment listed below to avoid contact with the liquid $N_2$. In case of contact treat minor wounds like burn wounds i.e. with luke warm water. Seek immediate medical assistance for treatment of larger wounds. All protective equipment as well as the measuring stick is available in the gas room*

*Materials:*
- thermo gloves
- closed shoes
- lab coat
- face shield
- measuring stick
- $N_2$ refill tank
- $N_2$ storage tank

- tube picker or tweezers

### 1.4.1 Refilling the $N_2$ storage tank

1. Measure the residual volume (cm) of $N_2$ in the tank with the measuring stick. Refill if the liquid level is lower than 12 cm from the middle metal platform.
2. Position the storage tank close to the outside door and the refill tank next to it so the refill tube can reach the storage tank.
3. Open the door.
   *NB: It will start beeping after some time. Press the key button alleviate this.*
4. Open the storage tank and place the muzzle of the tube coming from the refill tank a little bit down into the storage tank.
   *NB: Do not push the muzzle down too far since not completely cold gas will exit first which would warm up the tank interior and create a lot of mist.*
5. Open the tap to the refill tank tube and listen to the gas coming out. The sound changes when the tube is cold enough for the liquid nitrogen to go through.
6. When the sound changes move the muzzle deeper into the tank and hold it there for approx. 30 seconds with a fully opened tap.
7. Close the tap, take the muzzle out and hang it back over the refill tank.
   *NB: Make sure the tube hangs securely on the tank.*
8. Measure the new liquid nitrogen level and repeat steps 4-7 if the level is not high enough.
9. Close the storage tank and reposition the tanks.

### 1.4.2 Adding and removing samples

*NB: As soon as the rack is out of the tank, work quickly to avoid heating up the samples. The temperature difference is nearly 300 °C.*

1. Open the tank and slowly take out the rack. Place it on a cart or eq. to allow easy access.
2. Remove the metal pin by pressing it up from the bottom.

   *NB: Do not remove thermo while removing the metal pin.*
3. Take out the box you are going add/remove your sample to/from and use tweezers or the tube-picker which came with the freezer boxes.
4. Reposition the box and make sure the metal pin is correctly in place before slowly lowering the rack back into the tank.
5. Measure the $N_2$ level with the measuring stick to make sure there is enough left. Refill the tank if the liquid level is lower than 12 cm from middle metal platform.

## 2. Plate Preparation

*NB 1: Always use plastic gloves and work in the sterile environment inside the LAF cabinet. Clean the cabinet with 70 % ethanol. Spray gloves, working surface, microscope, centrifuge, incubator etc. with 70 % ethanol if you suspect a minor contamination.*

*NB 2: Cell suspensions are to be thoroughly mixed before any transfer where a correct cell concentration is of importance.*

## 2.1 Coating Plates

*Materials:*
- fibronectin, 100 µg/ml (mix carefully)
- 1×PBS
- glass bottomed 96-well plate

1. Dilute the fibronectin to 12.5 µg/ml (4.2 ml PBS + 0.6 ml fibronectin for one plate). Store undiluted fibronectin at -20 °C.
2. Add 40 µl to each well.
3. Incubate for 1 hour at room temperature or at 4 °C for storage up to two weeks.

Table 1. Recommended volumes

| No. of plates | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| fibronectin | 0.6 | 1.1 | 1.6 | 2.1 | 2.6 | 3.1 |
| PBS | 4.2 | 7.7 | 11.2 | 14.7 | 18.2 | 21.7 |

## 2.2 Cell Counting

*Materials:*
- Neubauer counting chamber
- Millipore Scepter with 60 µl tip
- manual counter
- cell growth media or 1×PBS

### 2.2.1 Manual Counting: Neubauer Counting Chamber

1. Dilute a small volume of the cell suspension (5× - 20× dilutions will usually yield the optimal 30 - 100 cells/Neubauer square) with 1×PBS or cell medium.
   *Example:* Dilute 50 µl cell suspension with 200 µl growth media (5× dilution).
2. Put small drops of the dilution on the counting chamber mounting support and mount the cover slip (make sure there are visible liquid-ring patterns) (figure 1).
3. Add the sample (will be absorbed by capillary force).
4. Count the cells in square number 1 - 4 (and 5 if necessary) using a manual counter. The cells inside a square and on 2 of 4 sides should be counted (figure 2).
5. Calculate the average number of cells per square and multiply by 10 000 and the dilution factor to yield the cell concentration in cells/ml.

*Example:*

$$\acute{x} = 50\,;\, d = 5$$

$$c = \acute{x} \cdot d \cdot 10\,000 = 2\,500\,000\,\text{cells/ml}$$

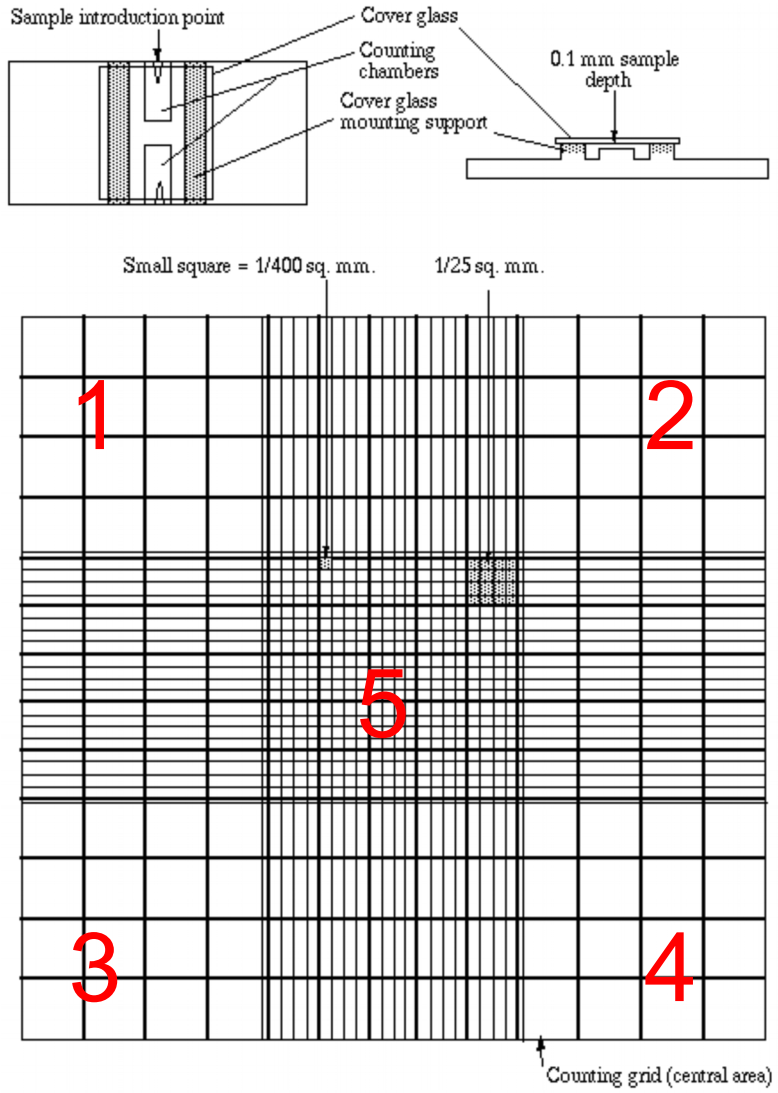


*Figure 1.* Schematic over Neubauer counting chamber.

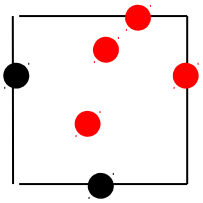

*Figure 2.* Count cells (red) inside and on 2 out of 4 sides in each square.

### 2.2.2 Automatic Counting: Millipore Scepter

1. Estimate the total number of cells by inspecting the Petri dish before detaching the cells. A confluent 10 cm dish contains ≤ 4 M cells and a ditto 15 cm ≤ 10 M.
2. Dilute the cell suspension to a concentration of 10 000 – 500 000 cells/ml with 1×PBS. Use a microcentrifuge tube to accommodate for the measuring tip

width. A sample volume > 100 µl is recommended (the counter will aspirate 50 µl).

*Example: Diluting for counting from a ~100 % confluent 15 cm Petri dish (~10 M cells) after detatching, centrifuging and resuspending in 1 ml media: 30 ul suspension + 970 ul PBS (33x dilution) yeilds a ~ 300 000 cells/ml dilution.*

3. Turn the cell counter on by pressing the toggle on the back and attach a 60 µl tip.
4. Press and hold down the plunger, submerge the tip and release the plunger while keeping the tip submerged i.e. follow the instructions on the Scepter screen.

*NB: Remember to multiply the measured concentration value by the appropriate dilution factor.*

## 2.3 Cell Seeding

*Materials:*
- 1×PBS + EDTA
- Trypsin (1×)
- fibronectin coated glass bottomed 96-well plate

### 2.3.1 Diluting the Cell Suspension

1. Detach and centrifuge the cell suspension to remove the trypsin (1700 rpm, 3 min) and resuspend the pellet in medium.
2. Determine the concentration by cell counting (section 2.2).
3. Calculate the volume of cell suspension needed i.e. $v_1$.

a) Number of cells per well see appendix
b) Number of cells per plate (i.e. $c_2v_2$)
c) Medium per plate (i.e. $v_2$) 80 µl × 125 wells = 10 000 µl
d) Calculating $v_1$
- equation: $c_1v_1 = c_2v_2$
- $$v_1\,(ml) = \frac{c_2v_2}{c_1} = \frac{2\,500\,000}{c_1}$$ (example: NIH-3T3)

4. Mix $v_1$ ml of cells with (10 - $v_1$) ml medium.

### 2.3.2. Manual Cell Seeding

1. Remove the fibronectin coating with a multi pipette or the 8-channel VacuBoy.

2. Add 80 µl of cell suspension to the center of each well without touching the bottom of the well with the pipet tip.
3. Remember to mix the cell suspension by pipetting before adding it to the wells as the cells can form aggregates and/or sediment.
4. Avoid shaking or tilting the plate and inspect it in the microscope to confirm even distribution of the cells.

5. Incubate in humidified air (37 °C, 5.2 % $CO_2$).

# 3 Primary Antibody Dilution

*Materials per portion:*
- 95 HPA antibodies
- 9.6 ml ml 1×PBS
- 400 µl FBS
- 10 µl anti-tubulin (cytoskeleton marker)
- 25 µl anti-KDEL (ER marker)
- 96-well PCR plate

*NB: The HPA antibodies are diluted in 1×PBS (4 % FBS) containing the anti-tubulin and anti-KDEL markers (a.k.a. stock solution). See section 5 for details on buffer preparation.*

## 3.1 Automated Dilution

- Dilutions from 384-well storage plates are done on the Tecan Freedom Evo.

1. Choose the amount of portions to be diluted and print the dilution work order and labels form LIMS.
2. Retrieve all needed 384-well plates from the freezer and allow them to equilibrate at temperature for at least 15 minutes.
3. Prepare stock solution as specified by the work order. Spin down the antibody marker tubes before opening.
   *NB: Be sure to remove any bubbles from the buffer tubes as they might otherwise cause incorrect automatic pipetting.*
4. Briefly spin down the 384-well plates in the plate centrifuge.
5. See the Tecan Freedom Evo SOP for instructions on how to run the liquid handler.

# 4 Immunostaining

*NB: See section 5 for details on buffer preparation. Volumes given below are per well and all unspecified incubation temperatures are 20 °C.*

## 4.1 Day 1

*Materials:*

- 1×PBS
- PFA (4 %)
- 1×PBS + 0,1 % Triton X-100
- primary antibody template dilution

1. *Fixation*
   a) Remove growth medium i.e. aspirate ~ 100 µl from all wells.
   b) Wash the cells once with 40 µl 1×PBS.
   c) Fix the cells by incubating in 40 µl ice cold PFA for 15 minutes.
2. *Permeabilization*
   a) Remove the PFA and permeabilize the cells by incubating with 40 µl 1×PBS + 0.1 % Triton X-100 for 3 × 5 minutes.
   b) Wash the cells once with 40 µl 1×PBS.

3. *Primary Antibody Incubation*
   a) Add 40 µl primary antibody and incubate over night at 4 °C or in room temperature for 2 h.

## 4.2 Day 2

*Materials:*

- 1×PBS
- blocking buffer (1×PBS + 4 % FBS with secondary antibodies)
- DAPI
- glycerol (5 % 10×PBS)
- 96-well PCR plates

1. *Secondary Antibody Incubation*
   a) Remove the primary antibody and wash the cells with 40 µl 1×PBS for 4 × 10 minutes.
   b) Add 40 µl blocking buffer with secondary antibody and incubate for 90 minutes.
2. *DAPI Nuclear Staining and Mounting*
   a) Remove the blocking buffer and incubate in 40 µl DAPI for 10 minutes.
   b) Wash the cells with 40 µl 1×PBS for 4 × 10 minutes and mount with ~ 350 µl glycerol/10×PBS.

   c) Adjust the level of glycerol if needed and seal the plate with foil cover.

# 5. 384well Plate Preparation Preliminary

**Note:** The 16 well multi channel pipette can be found in the wet lab on the production bench. After you are done in the cell lab, please put it back there.

**Note:** The current 384well Greiner sensoplates come without a lid. Please take a lid from an old plate (either 96well or 384well) into the cell lab, disinfect it with ethanol thoroughly and use it for the new plate. Because of this reason, please avoid writing directly on the lid. Use tape instead.

**Note:** Due to the limited travel range of the microscope stage, we are forced to skip column 1 and row P. THE NEGATIVE CONTROL WILL BE IN WELL O24.

**Note**: Always try to pipet straight and close to the bottom in order to make sure that the coating reagents/cells/… reach the glass surface. Avoid pipetting onto the walls as much as possible!

## 5.1 Coating plates

1. Dilute the fibronectin to 12.5 µg/ml (1:8 dilution; 7.35 ml PBS + 1.05 ml fibronectin for one plate). Store undiluted fibronectin at -20 °C.

2. Add 20 µl to each well.

3. Incubate for 1 hour at room temperature or at 4°C for storage up to two weeks.

## 5.2 Cell seeding

**NOTE:** total volume/well = 40 µl, cell numbers in production Master sheet on Google Drive.

1 Remove the fibronectin coating with a multi pipette or the 8-channel VacuBoy.

2 Add 40 µl of cell suspension to the center of each well without touching the bottom of the well with the pipette tip.
NOTE: pipet straight and make sure that the cell suspension reaches the glass surface

3 Inspect the plate under the microscope to confirm an even distribution of the cells.

4 Let the plate stand on the bench in RT for ~10 minutes to allow the cells to settle down, before placing it in the incubator.

5 Incubate in humidified air (37 °C, 5.2 % CO2).

## 5.3 Antibody dilution

Prepare at least 20 µl of antibody dilution in the 384well “reagents” plate (square wells; smaller than antibody storage plate).

### 5.3.1 Automated antibody dilution:

**NOTE:** the antibody dilution is carried out on the freedom evo pipetting robots. Load the worklist from LIMS. The script is similar to the dilution for 96 well format, however besides the target plate (384well reagents) a 96well standard PCR plate is needed for a predilution (1:30) of highly concentrated antibodies.

1. Pipet 29 µl of antibody stock buffer into the wells of the 96well PCR plate, that are needed for serial dilution of antibodies.

2. Run the script.

3. Spin down the 96well PCR plate before the antibodies will be transferred to the 384 well plate. There is a user prompt at the robot, stopping the program until this step is done.

## 5.4 Immunostaining

### 5.4.1 Day 1

**NOTE:** carried out on the Fluent pipetting robot; please use the latest script(s).

**For one plate:**
Prepare 100 µl/well of PBS & Triton in a 384well plate (“ab storage plates”). There is a pre Day 1 script available on the freedom evo pipetting robot (T2)
Prepare 20 µl/well of PFA in a 384well reagents plate.

**OBS: SPIN DOWN ALL REAGENTS BEFORE USE!**

1. *Fixation*

Remove growth medium i.e. aspirate ~ 100 µl from all wells and wash the cells once with 20 µl 1×PBS.

Fix the cells by incubating in 16 µl ice cold PFA for 15 minutes.

2. *Permeabilization*

Remove the PFA and permeabilize the cells by incubating with 20 µl 1×PBS + 0.1 % Triton X-100 for 3 × 5 minutes.

Wash the cells once with 20 µl 1×PBS.

3. *Primary Antibody Incubation*

Add 16 µl primary antibody and incubate over night at 4 °C or in room temperature for 2 h

### 5.4.2 Day 2

**NOTE:** carried out on the Fluent pipetting robot; please use the latest script(s).

**For one plate:**
Prepare 150 µl/well of PBS & Glycerol in a 384well plate (“ab storage plates”). There is a pre Day 2 script available on the freedom evo pipetting robot (T2).
Prepare 20 µl/well of DAPI and 2$^{nd}$ antibodies in a 384well plate (“reagents”)

**OBS: SPIN DOWN ALL REAGENTS BEFORE USE!**

1. *Secondary Antibody Incubation*

Remove the primary antibody and wash the cells with 20 µl 1×PBS for 4 × 10 minutes.

Add 16 µl blocking buffer with secondary antibody and incubate for 90 minutes.

2. *DAPI Nuclear Staining and Mounting*

Remove the blocking buffer and incubate in 16 µl DAPI for 10 minutes.

Wash the cells with 20 µl 1×PBS for 4 × 10 minutes and mount with ~120 µl glycerol/10×PBS.

Adjust the level of glycerol if needed and seal the plate

# 6 Buffers and Solutions

*NB 1: Always use pressure safe 1l bottles when vacuum filtering buffers. The filtering pressure flow is to be turned on before attaching the tubing to the filtering casette.*

*NB 2: When autoclaving buffers, the caps are to be on the bottles but not screwed on. Label the autoclaving tape and attach it to the cap (not the bottle). Always place the autoclave thermometer in a liquid containing flask together with your buffers when autoclaving.*

## 6.1 PBS

**10×PBS**

- 160 g NaCl
- 4 g KCl
- 28.8g $Na_2HPO_4$
- 4.8 g $KH_2PO_4$

- Dissolve in 1600 ml Milli-Q. Adjust pH to 7.2 and add Milli-Q up to two liters, vacuum filtrate and autoclave the buffer.

**1×PBS**

- Mix 100 ml 10×PBS with 900 ml Milli-Q. Autoclave.

**1×PBS + EDTA**

- Mix 0.37 g EDTA with 100 ml 10×PBS and 900 ml Milli-Q. Autoclave the buffer.

## 6.2 Fibronectin

*NB: The following steps are to be performed in a LAF cabinet.*

1. Equilibrate the fibronectin in room temperature.
2. Add 5 ml Milli-Q to one bottle of 5 mg lyophilized fibronectin and incubate for 30 minutes. Do not shake or mix more than absolutely necessary.
3. Add 45 ml Milli-Q and aliquot in Eppendorf tubes (1.1 ml/tube) marked with “Fn”.
4. Store the diluted fibronectin in the AC-lab freezer.

### 6.3 PFA (Paraformaldehyde) 4 %

*NB:* Remember the following safety procedures when working with PFA. Use blue gloves, lab coat and eye protection and work in a fume hood or specifically ventilated lab area. Use mouth guard when working with the powder. Residues are collected in a special container and sent for destruction. Smaller amounts in micro plates can be wrapped in Parafilm and thrown in the regular bins. Always make larger amounts of PFA (about 150 ml) and freeze in skirted PCR plates (e.g. a few 50 µl and 150 µl plates and a lot of 180 µl plates).

1. Pre heat 150 ml of any cultivation medium with 10 % FBS in a fume hood, on a hot plate magnetic stirrer to 60° C using a ≥ 250 ml beaker (set the heating plate to ~120° C).
2. Add either 6 g of PFA or 50 ml of the liquid PFA-stock to the warm growth medium and leave stirring for ca. 20 min until the powder dissolves or the stock is mixed.
3. Add concentrated NaOH until the solution is and all the PFA is fully dissolved (pH ~11).
4. Allow the PFA to cool down to room temperature and set pH to 7.2 - 7.3. Use diluted HCl for the adjustment.
5. Transfer 50 µl, 100 µl, 150 µl and180 µl, to microtiter plates for preparation of 1, 2, 3 and 4 plates respectively.

   *N.B. If you have been forced to adjust the pH several times, discard the PFA and prepare a new solution.*

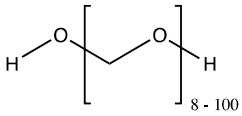


*Figure 3.* PFA

### 6.4 PBS with 0.1 % Triton X-100

*NB 1: When making Triton solution on Mondays, be sure to make enough for all plate preps that are to be made during the coming week. The volumes below are for mixing Triton solution for three plates. Do not use Triton solutions older than 7 days.*

*NB 2, Dissolving Triton: Use the 5 ml pipette, set to maximally 3 ml, and avoid getting to much undissolved Triton into the tip (especially since this often cause a lot of foam formation).*

1. Mix 50 µl Triton X-100 with 5 ml PBS.
2. Pipette carefully otherwise it will build up foam.
3. When it is dissolved add another 45 ml of PBS.

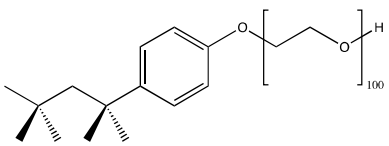

*Figure 4.* Triton X-100

### 6.5 Stock Solution - Blocking Buffer (PBS + 4 % FBS) with Primary Antibodies

1. 9.6 ml PBS
2. 400 µl FBS

3. 10 µl anti-tubulin
4. 25 µl anti-KDEL

Table 2: Primary antibody details

| Manufacturer | id. no. | origin | affinity | c [mg/ml] | dil. c [µg/ml] |
|---|---|---|---|---|---|
| Abcam | ab7291 | Ms mAb | alfa-tubulin | 1 | 1 |
| Abcam | Ab50601 | Rt mAb | KDEL | 1 | 2.5 |
| HPA | - | Rb pAb | PrEST:s | 0.1 – 1.5 | 2 – 4 |

### 6.6 Blocking Buffer (PBS + 4 % FBS) with Secondary Antibodies

- The secondary antibodies are diluted 800x in PBS + 4% FBS.

- For one plate (4 ml) mix:

1. 3840 µl PBS + 160 µl FBS
2. 5 µl of Alexa 555
3. 5 µl of Alexa 647
4. 5 µl of Alexa 488

*NB 1: Do not mix or go down to deep with the pipette tip in the Alexa vials. After mixing the solution, aliquot 40 µl/well in a 96-well skirted PCR plate.*

*NB 2: The Alexa Fluor antibodies are photosensitive. Cover tubes and plates with aluminum foil and turn off the lights in the laboratory if possible.*

*NB 3: If the electric multi pipette is to be used for dispensing the antibodies, it is better to prepare a slightly larger volumes (Table 3).*

Table 3: Volumes to be mixed when dispensing with electronic multi pipette

| No. of plates | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| PBS [µl] | 4 032 | 8 064 | 12 096 | 16 128 | 20 160 |
| FBS [µl] | 168 | 336 | 504 | 672 | 840 |
| Alexa 488 [µl] | 5.25 | 10.5 | 15.75 | 21 | 26.25 |
| Alexa 555 [µl] | 5.25 | 10.5 | 15.75 | 21 | 26.25 |
| Alexa 647 [µl] | 5.25 | 10.5 | 15.75 | 21 | 26.25 |
| *vol mix per well [µl]* | *40* | *80* | *120* | *160* | *120 + 80* |

Table 4: Secondary antibody details

| Manufacturer | id. no. | abs. λ [nm] | origin | affinity | c [mg/ml] | dil. c [µg/ml] | emit λ [nm] |
|---|---|---|---|---|---|---|---|
| Invitrogen | A11034 | 488 | Gt | Rb | 2 | 2.5 | 519 |
| Invitrogen | A21424 | 555 | Gt | Ms | 2 | 2.5 | 565 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Invitrogen | A21449 | 647 | Gt | Chk | 2 | 2.5 | 665 |

### 6.7 DAPI (4', 6-diamidino-2-phenylindole)

*NB: DAPI is photosensitive. Cover tubes and plates with aluminum foil and turn off the lights in the laboratory if possible.*

*Pre-Dilution*: (only if the pre-diluted ones are out) Mix 80 µl 14.3 mM DAPI with 920 µl Staq in a 1.5 ml microcentrifuge tube. Aliquot 35 µl into 100 µl microcentrifuge tubes and store these in a 50 ml Falcon tube.

1. Mix pre-diluted DAPI with PBS (Table 5).
2. Put 50/100/150 µl/well in a skirted PCR plate for preparing 1 - 5 plates.

Table 5: DAPI dilution

| No. of plates | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| PBS [ml] | 5.5 | 11 | 16.5 | 22 | 27.5 |
| DAPI [µl] | 11 | 22 | 33 | 44 | 55 |
| *vol dil per well [µl]* | *50* | *100* | *150* | *200* | *150 + 100* |

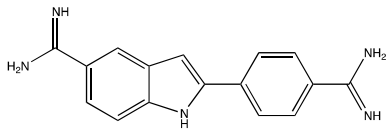


*Figure 5*. DAPI

### 6.8 Glycerol + 10×PBS

- For one plate:

1. 45 ml glycerol
2. 5 ml 10×PBS
3. Mix thoroughly in a 50 ml Falcon tube.

*NB: To continually keep a stock of ready to use glycerol, prepare 1 l bottles. I.e. mix 900 ml glycerol with 100 ml 10×PBS, autoclave and label the lid (content, initials, autoclavation date).*

## 7 Cell Line Details

*N.B. Figures for full plates (column 4) below are for 10 ml i.e. 125 wells at 80 ul.*

| cell line | medium | cells/well | cells/plate (min/ON) | time [h] (min/ON) | comment |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| A431 | RPMI, 10% FBS | 15 000 | 1 875 000/<br>1 000 000 | 8 / 20 | |
| A549 | DMEM, 10% FBS | 15 000 | 1 875 000/<br>1 000 000 | 4 / 20 | |
| BJ | DMEM, 10% FBS | 10 000 | 1 250 000 | 20 | |
| CaCo2 | EMEM, 10% FBS, 1% NEAA, 1% L-glut | 22 000 | 2 750 000 | 20 | wash thoroughly |
| HaCaT | DMEM, 10% FBS, 1% L-Glut | 5 000 | 625 000 | 20 | wash thoroughly |
| HeLa | EMEM, 10% FBS, 1% NEAA | 12 000 | 1 500 000 | 4 | |
| Hek-293 | EMEM, 10%, 1% NEAA | 22 000 | 2 750 000 | 20 | |
| Hep-G2 | EMEM, 10% FBS, 1% NEAA, 1% L-glut | 22 000 | 2 750 000 | 20 | |
| HTC | EMEM, 10% FBS, 1% NEAA | 18 000 | 2 250 000 | ? | |
| HUV-EC-C | Kaighn's,<br>10% FBS, 1% ESGS,<br>1% hepavin | | | | testing amount for 3 day seed, like BJ. count everything |
| MCF-7 | EMEM, 10% FBS, 1% NEAA, 1% L-glut | 8 000<br>(17 000) | 1 000 000<br>(2 125 000) | 20 | wash thoroughly small, count all |
| NIH 373 | DMEM, 10% FBS | 15 000 | 1 875 000 | 4 | |
| NTERA | DMEM, 10% FBS,<br>5 % HS (horse sera),<br>1 % L-glut | 7000 | 875 000 | 20 | |
| PC-3 | Kaighn's, 10% FBS | 17 000 | 2 125 000 | 20 | |
| RH-30 | RPMI, 10% FBS | 20 000/<br>18 000 | 2 500 000/<br>2 250 000 | 4 / 20 | (evaluate again) |
| RT-4 | McCoy's, 10% FBS | 24 000 | 3 000 000 | 20 | small, count all |
| SiHa | RPMI, 10% FBS | 20 000/<br>12 000 | | 4 / 20 | |

| **cell line** | **medium** | **cells/well** | **cells/plate (min/ON)** | **time [h] (min/ON)** | **comment** |
|---|---|---|---|---|---|
| SH-S5SY | DMEM, 10% FBS | (8 000) /<br>10 000 | (1 000 000) /<br>1 250 000 | (10) / 20 | |
| SK-MEL 30 | RPMI, 10% FBS | 15 000/<br>10 000 | 1 250 000 /<br>1 875 000 | 4 / 20 | |

| | | | | |
|---|---|---|---|---|
| U-2 OS | McCoy's, 10% FBS | 15 000 | 1 875 000/<br>1 000 000 | 4 / 20 |
| U251 | EMEM, 10% FBS | 15 000 | 1 875 000/<br>1 000 000 | 4 / 20 |
| Vero | RPMI, 10% FBS | 13 000 | 1 625 000 | 4 |

# 8 Microscope Rooms

## 8.1 General Microscope Care and Cleaning

1. When not in use, keep microscopes protected with the plastic cover.

2. Regularly remove dust from microscope table, stage, body and other surfaces where dust settles with a ethanol-damp cloth.

3. Accumulated dust within a microscope can deteriorate image quality. Keep all openings covered with dust caps so that dust does not enter the microscope and settle on inaccessible lenses, mirrors, and prisms.

4. Gently clean the ocular front lens with lens tissue if necessary. Do not touch, or clean, the ocular back lens if not absolutely necessary.

5. Avoid imaging badly sealed plates. Glycerol can drip down onto the stage or into microscope interior, collector lens etc.

## 8.2 Objectives

1. Always check which objectives are mounted on the microscope turret before starting imaging.

2. Avoid using the 63x oil with the 40x attached to the turret at the same time. This is to avoid increasing the risk of collisions with the galvo stage motor when stages move sideways.

3. Never apply strong physical force to an objective.

4. To switch objectives, rotate the turret using the touchscreen controls, LAS-AF or by *gently* rotating the turret manually.

5. Remove/install objectives using both hands. Loosely grip with one hand and twist the barrel with the other, being very careful not to touch the front lens with your fingers.

6. Take extreme caution not to drop the objective.

7. Store objectives in their respective labeled storing cylinder and always replace the turret dust caps after having removed an objective.

### 8.3 Objectives - cleaning

1. Excess oil should immediately be wiped away with ethanol damp lens tissue. Excess oil attract dust and can drip down onto the stage or condenser lens.

2. If possible wipe the perimeter around the front lens before wiping the actual front lens. This is to avoid dragging any unnecessary dust or particles onto the front lens.

3. Do not clean or touch the objective back lenses unless absolutely necessary.

4. Use each side of a lens tissue only once.

5. Never apply strong physical force when cleaning objectives.

6. Take extreme care to never get immersion oil on an air objective. This can happen inadvertently when rotating air objectives across an oil-coated slides/plates. Air lenses are not designed to be immersed and can be seriously damaged by oil or other immersion liquids.

7. An occasional thorough cleaning of the top lens on immersion objectives is necessary. Avoid doing this more often than necessary, as cleaning agents and cleaning erode an objective's front lens coating over time. Dried immersion liquids may need 100 % ethanol to be properly soluted and removed.