QPatch Compact

Guide for manual preparation of cells and solutions

Preparing cells

The success rate for experiments on QPatch Compact highly depends on the cell quality. The optimal harvesting procedure for obtaining viable and single cells in suspension must be determined for each cell line.

Optimal cells for your experiments start with proper cell culturing. Please refer to the Sophion Application Report: Cell culturing for automated patch clamp to get in-depth details on cell culturing (Included in hard copy).

Materials

- PBS
- Detachin®
- Suspension media (SFM)
    - (EX-CELL® ACF CHO medium)
    - 25mM HEPES
    - 100 U/mL penicillin/streptomycin
    - If using trypsin for detachment, add 0.04 mg/mL Soybean Trypsin Inhibitor
- Cell counter (Manual or automated)
- Cell viability measure (e.g. Trypan blue staining assay)
- Low speed and low volume centrifuge
- Pipettes and pipette tips (Covering 0.5 to 10 ml)

How to gently harvest cells and ensure high cell viability:

- Remove and discard the supernatant and gently wash the adherent cells with approx. 10 ml PBS (T175 flask). Tip the flask to ensure a gentle washing of the whole cell layer. Repeat washing twice.
- Remove all the PBS and add detaching agent (Sophion recommends using 3 ml Detachin®). Overflow the cells a couple of times before removing the detaching agent leaving 0.5 mL in the cell flask.
- Place the culture flask in the incubator at 37°C for 5 minutes to loosen the cells from the surface. Do not disturb the cells by tapping the culture flask. Add approx. 5 ml suspension media depending on your cell confluency (T175 flask) and pipette up and down 4-5 times to gently separate the cells. Preferably, the cell density of the suspension should be around 2-5 million cells/mL. Ensure single cells suspension.
- Count the cells and check the cell viability (should be above 95%).
- The cells can be kept in a stirrer or on a rocking table until they are used for experiments. The cell suspension can last between 4-8 hours depending on the cell type.

Improving cell viability

Prior to starting the experiments, cells must be washed and prepared in extracellular solution. Some cell types are more fragile and can easily be damaged during centrifugation.

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How to wash cells to remove cell debris and dead cells:

- Add 0.5 ml cell suspension to an Eppendorf tube and spin the cells in a centrifuge. Centrifugation force and time must be determined for each cell line. Sophion recommends:

  CHO cells:

  - Centrifugation centripetal force: 100 G
  - Centrifugation time: 3.5 minutes

  HEK cells:

  - Centrifugation centripetal force: 80 G – 100 G
  - Centrifugation time: 3.5 - 4.5 minutes

- Remove the supernatant and wash the cells in 1 ml extracellular saline solution. Spin the cells down again and remove the supernatant.
- Resuspend the cells in a volume that corresponds with the desired cell density. For each site of the QPlate for the QPatch Compact, 5µL cell suspension is needed.

Ensuring optimal cell density

The optimal cell density depends on the cells type used in the experiment. A lot can be gained by adjusting the concentration of the cells to ensure a high success rate. Sophion recommends the following densities of your final cell preparation:

CHO cells:

- 4-6 x 10^6/ml

HEK cells:

- 6-8 x 10^6/ml

The recommended cell density is for both single- and multi-hole QPlates.

The cells should be applied to the QPatch Compact immediately after preparation.

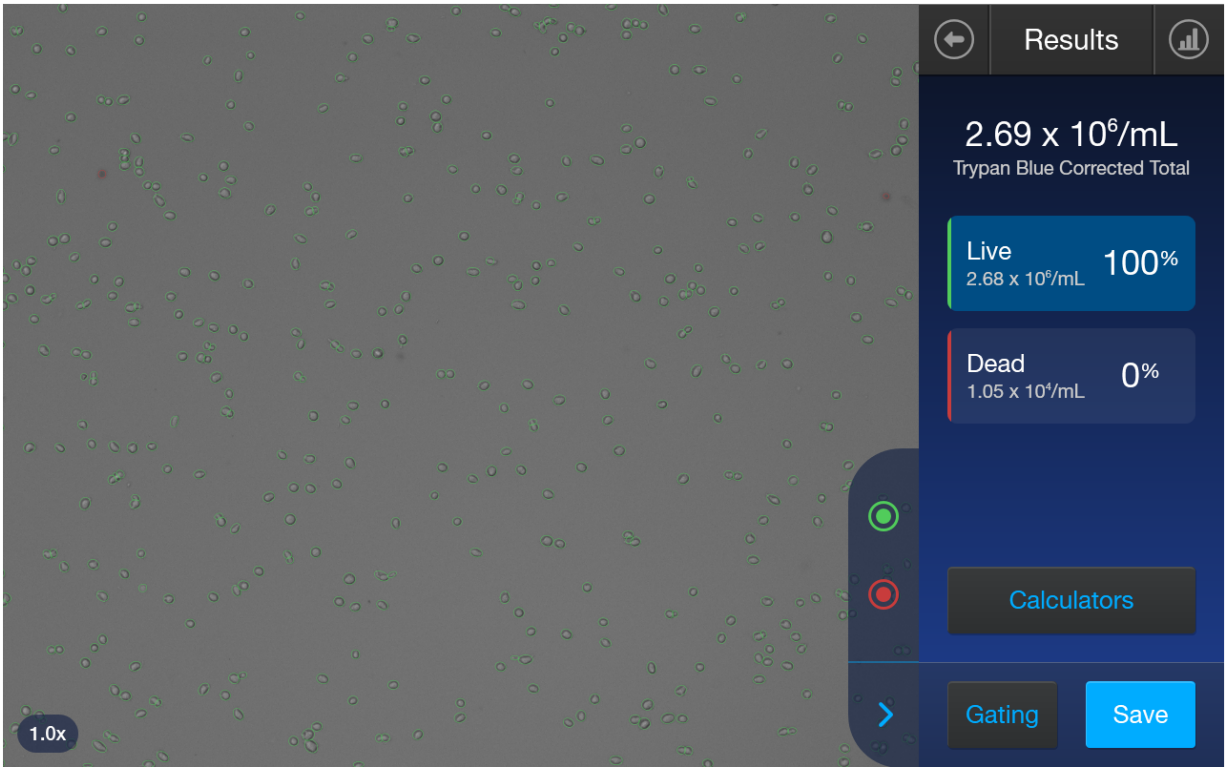

Figure 1 - Example of good cell viability and cell density. The figure shows a cell count of CHO cells in suspension using the Countess™ 3 Automated Cell Counter. All types of automated and manual cell counters can be used for counting. It is preferred if the viability can also be measured.

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Guide for preparation of cells and solutions

Preparing intra- and extracellular solutions

Careful preparation of solutions is key for successful experiments.

While you are most welcome to use your own intracellular and extracellular solution compositions, the recipes below will likely cover most of your needs.

QPatch Compact can, like the other QPatch solutions, run with gigaOhm seal performance in physiological solutions. However, you can use fluoride containing intracellular solutions or other seal enhancing agents, as you see fit depending on your assay needs.

Special care is to be taken when:

Solutions contain glucose:

- Add glucose prior to experiment or to solutions used within the next few days
- Do not filter after glucose addition

Solutions contain ATP:

- Add ATP immediately before an experiment (ATP degrades over the course of the day)
- Remember to adjust the pH after ATP addition
- Do not use ATP in fluoride containing intracellular solutions, as the two precipitate.

Solutions contain IP_3:

- Add IP_3 immediately before an experiment
- IP_3 degrades at 4°C and is light sensitive.
- Preparation:
    - 1) Dissolve glutamate and EGTA in CsOH
    - 2) Add stock solution
    - 3) Adjust pH

Storage

Open bottles stored at room temperature should be used within 6 months.

Stock stability

Stock solutions must be stored in the refrigerator.

Stock solutions may be stored for a maximum of 3 months, except HEPES and Glucose, which may only be stored for one month.

ATP and IP_3 must be stored in the freezer (stable at room temperature for one day).

Do not filter solutions containing glucose.

IC000 (KCl containing intracellular solution)

		1000 ml	
	Total concentration	Mass	Stock conc. – Volume
$CaCl_2$	5.374 mM	0.79 g	1 M – **5.37 ml**
$MgCl_2$	1.75 mM	0.356 g	1 M – **1.75 ml**
KOH/EGTA	31.25 mM//10 mM	1.75 g/3.8 g	0.25/0.08 M – **125 ml**
HEPES	10 mM	2.383 g	1 M – **10 ml**
KCl	120 mM	8.946 g	2 M – **60 ml**
Na_2–ATP	4 mM	2.204 g	
pH = 7.2 with KOH, Osmolarity = 285 – 296 mOsm with sucrose (Before adjustment 270 – 295 mOsm) free Ca=115 nM and free Mg=137 µM			

NOTE*: Ensure to check osmolarity after preparation

IC500 (CsF containing intracellular solution)

		1000 ml	
	Total concentration	Mass	Stock conc. – Volume
CsF	140 mM	21,26 g	1 M – 140 ml
EGTA/CsOH	1 mM / 5 mM	-	0.05/0.25 M – 20 ml
HEPES	10 mM	-	1 M – 10 ml
NaCl	10 mM	584.4 mg	584.4 mg
pH = 7.3 with 3M CsOH, Osmolarity = 320 mOsm with sucrose (Before adjustment 280 – 290 mOsm)			

NOTE*: Ensure to check osmolarity after preparation

IC700 (KF containing intracellular solution)

		1000 ml	
	Total concentration	Mass	Stock conc. – Volume
KF	120 mM	6,972 g	2 M – 60 ml (6,972 g)
KCl	20 mM	1,492 g	2 M – 10 ml
HEPES	10 mM	-	1 M – 10 ml
EGTA	10 mM	3,8 g	3,8 g
pH: 7.2 mOsm with KOH, Osmolarity = 300 mOsm with sucrose			

NOTE*: Ensure to check osmolarity after preparation

EC000 (NaCl containing extracellular solution)

		1000 ml	
	Total concentration	Mass	Stock conc. - volume
CaCl2	2 mM	0.294 g	1 M – 2 ml
MgCl2	1 mM	0.203 g	1 M – 1ml
HEPES	10 mM	2.383 g	1 M 10 ml
KCl	4 mM	0.298 g	2 M – 2 ml
NaCl	145 mM	8.474 g	8,474 g
Glucose	10 mM	1.802 g	1 M – 10 ml
pH = 7.4 with NaOH, Osmolarity = 305 mOsm with sucrose (Before adjustment 285 – 295 mOsm)			

NOTE*: Ensure to check osmolarity after preparation