QPatch Compact

Guide for 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, starts 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
- Cell counter (Manual or automated)
- Cell viability measure (e.g. Trypan blue staining assay)
- 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 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 2.5 ml detaching agent.
- 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 5 ml suspension media[1] (T175 flask) and pipette up and down 4-5 times to gently separate the cells. 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 lasts between 4-8 hours depending on the cell type.

[1] Suspension Media (SFM): Serum Free Medium (Sigma C5467), 25 mM HEPES and 0.04 mg/ml Soy Bean Trypsin Inhibitor.

Improving cell viability

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

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 concentration.

Ensuring optimal cell density

The needed 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:

CHO cells:

- 4-6 x 10^6/ml, ie. 2 x 10^4- 4 x 10^4 cells per site

HEK cells:

- 1-3 x 10^6/ml, ie. 5 x 10^3-1,5 x 10^4 cells per site

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

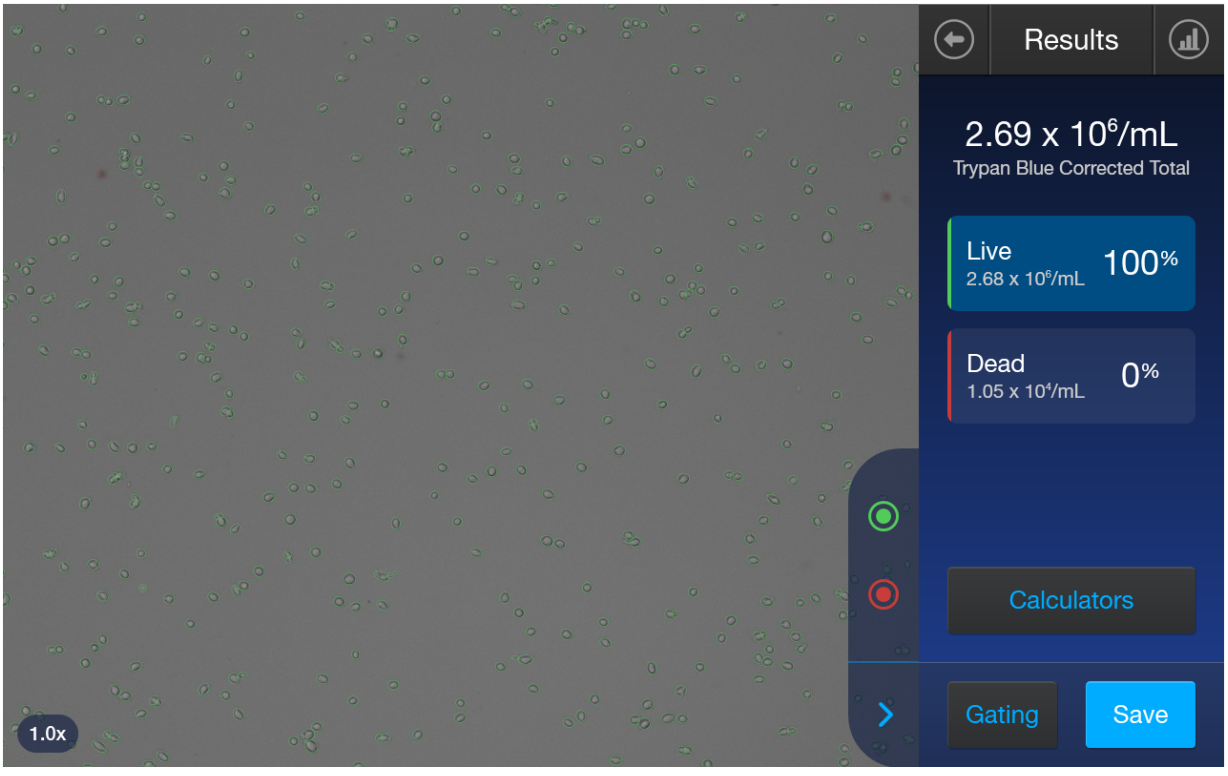

Figure 1 Good example of cell viability and cell density

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Guide for preparation of cells and solutions

Preparing IC and EC solution

Careful preparation of Ringer's solution is key for successful experiments.

While you are most welcome to use own IC and EC compositions, below IC and EC recipes will likely cover most of your needs.

QPatch Compact can, as the other QPatch solutions, run with gigaOhm seal performance in physiological solutions, however you can use fluoride containing ringers 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

Solutions contain ATP:

- Add prior to experiment (ATP degrades over the course of the day)
- Remember to adjust the pH after ATP addition

Solutions contain IP_3:

- Add prior to 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 max be stored for 3 months, except HEPES and Glucose, which may only be stored for one month.

IC000 KCl–Ringer's solutions

		1000 ml	
	Total concentration	Mass	Stock conc. – Volume
$CaCl_2$ $MgCl_2$ KOH/EGTA HEPES KCl	5.374 mM 1.75 mM 31.25 mM//10 mM 10 mM 120 mM	0.79 g 0.356 g 1.75 g/3.8 g 2.383 g 8.946 g	1 M – **5.37 ml** 1 M – **1.75 ml** 0.25/0.08 M – **125 ml** 1 M – **10 ml** 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 Cesium–Fluoride Ringer

		1000 ml	
	Total concentration	Mass	Stock conc. – Volume
CsF EGTA/CsOH HEPES NaCl	140 mM 1 mM / 5 mM 10 mM 10 mM	21,26 g - - 584.4 mg	1 M – 140 ml 0.05/0.25 M – 20 ml 1 M – 10 ml 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–Ringer

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

NOTE*: Ensure to check osmolarity after preparation

EC000 NaCl–Ringer's solutions

		1000 ml	
	Total concentration	Mass	Stock conc. - volume
CaCl2 MgCl2 HEPES KCl NaCl	2 mM 1 mM 10 mM 4 mM 145 mM	0.294 g 0.203 g 2.383 g 0.298 g 8.474 g	1 M – 2 ml 1 M – 1ml 1 M 10 ml 2 M – 2 ml 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