Rescuing APC assay on low expressing cell line using fluorescence-based cell sorting

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Introduction

$K_V1.3$ is a voltage-gated K^+ channel from the Shaker-related subfamily that plays a key role in T cell proliferation and activation by regulating their membrane potential and Ca^{2+} signaling, making it a promising therapeutic target for autoimmune disorders. In addition to its role in immune function, $K_V1.3$ is highly expressed in various cancer cells, where it may promote tumor growth, and it is also linked to inflammation-driven neurological diseases. Given its broad relevance, modulating $K_V1.3$ pharmacologically holds significant potential for developing new treatments. High expression of $K_V1.3$ in recombinant cell lines is therefore critical for ensuring reliable, reproducible and efficient drug screening in Automated Patch Clamp (APC) assays.

In this study, we applied fluorescence-activated cell sorting (FACS) to enrich the population of cells expressing $K_V1.3$, aiming to enhance the success rate, reliability and reproducibility of APC experiments. Live CHO- $K_V1.3$ cells were stained with a PE-labelled antibody targeting the extracellular domain of $K_V1.3$. Sorted cells were selected based on fluorescence intensity and subsequently tested on a QPatch system.

Our results showed that sorting of CHO-$K_V1.3$ cells based on channel expression significantly improved APC assay success rates, from an average of 20% to 80%, without compromising cell viability, seal formation, or ion channel activity. This approach demonstrates that FACS-based sorting can rescue APC assays with low-expressing cell lines, offering a robust method for improving throughput and reliability in ion channel drug discovery.

Conclusion

With this study, we show that sorting CHO-K_V 1.3 cells based on channel expression prior to APC experiments can substantially increase the final success rate (from an average of 20% to an average of 80%). This increase can be achieved without significantly affecting the quality of CHO-K_V 1.3 cells in terms of viability, seal formation and channel activity.

This report demonstrates how an APC assay on a low expressing cell line can be rescued by using fluorescence-based cell sorting.

Material and methods

CHO-K_V 1.3 cells were cultured following Sophion SOP for CHO cell line. On the day of the experiment, cells were harvested from the culture flask and counted before starting with the staining procedure. Cell staining was performed following the manufacturer's SOP.

Flow cytometry and cell sorting:
Flow cytometry analysis was performed using the MacsQuant Analyzer 10 (Miltenyi Biotec). Specifically, the PE fluorescence signal was recorded in the B2 channel (Excitation: 488 nm, Filter: 585/40 nm). Based on the results obtained with the flow analysis, the stained cells were successively sorted using the MACSQuant Tyto Cell Sorter (Miltenyi Biotec).

APC tests:
All experiments were carried out at room temperature using Sophion QPatch and QPlate single-hole consumables. Cells were patched using a standard whole cell protocol and standard $K_V1.3$ ringers.

Data analysis:
Analysis was performed using the Sophion Assay Software, GraphPad Prism 7.03 (GraphPad Software Inc.) and MACSQuantify software (Miltenyi Biotec).

Results

Cell staining and flow cytometry analysis

To ensure the quality and specificity of $K_V1.3$ staining, we analyzed samples using the MACSQuant Analyzer10 (Miltenyi Biotec) before sorting. We confirmed that the $K_V1.3$-positive fraction could be distinguished from unstained cells. The stained sample (Fig.1, blue) was compared to non-stained cells (Fig.1, control 1 - red) and cells treated with only the secondary antibody (Fig.1, control 2 - green) to check for unspecific binding.

The flow cytometry analysis indicates that the staining process was successful, as part of the cell population of the stained sample shows fluorescence (Fig.1, blue histogram), while no fluorescent signal is recorded from the two control samples (Fig.1, red and green histograms). Specifically, in the example shown in Fig.1, the fraction of cells expressing $K_V1.3$ ion channel corresponds to about the 11% of the entire cell population.

Sorting of $K_V1.3$-expressing cells and QPatch test:
The fraction of cells with high $K_V1.3$ expression was isolated using the Tyto cell sorter (MACSQuant Tyto, Miltenyi Biotec) and collected for APC experiments. Sorted cells were then tested on QPatch to measure the level of $K_V1.3$ current. The quality filters used for the analysis were: $I_{Kv1.3}$ >400pA, C_{slow} >4pF, R_{mem} >200MΩ. Representative current traces recorded from a cell with high $K_V1.3$ expression and from a cell with low $K_V1.3$ expression, respectively, are reported in Fig. 2.

To ensure that the staining process did not negatively affect the cell viability or performance, cells that were not stained but went through the staining steps (using only buffer without antibodies) and cells that were stained but not sorted were tested in comparison to the stained and sorted sample. Likewise, to test the impact of sorting, freshly harvested cells and cells collected from the negative chamber of the sorting cartridge were tested as well (Fig. 3).

The histogram shows that freshly harvested cells had a success rate of about 16%, while unstained and unsorted stained cells did not exceed 25%. In contrast, cells that were stained and sorted achieved a success rate above 80%. This significant increase is solely due to ion channel expression, as the staining and sorting process did not affect the final count of completed experiments (Table 1, Fig. 4).

The success rate of the experiment with cells collected from the negative chamber of the sorting cartridge, being only 5%, gives a good indication of the quality of the staining and sorting process.

Reculture of sorted cells:
Sorted cells were re-cultured to investigate whether they could maintain the high $K_V1.3$ expression rate, and they were successively tested on QPatch, at different time points (Fig. 5). From fig. 5 it is possible to see that the success rate of QPatch tests with cells re-cultured and harvested at different time points, decreases over time. However, it is possible to maintain a success rate above 50% for up to one week after sorting. This enables experiments on these cells without requiring the long staining and sorting process right before performing APC tests.

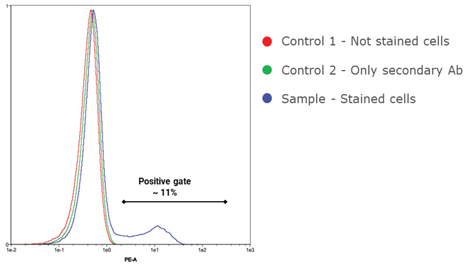

Fig. 1: Representative flow cytometry histograms: Blue - stained sample where, starting from left to right, the first peak corresponds to the $K_V1.3$ negative cells and the second peak corresponds to the $K_V1.3$ positive cell fraction, hence showing PE fluorescence. Red - control 1, corresponding to non-stained cells. Green - control 2, corresponding to cells treated with only the secondary antibody, hence indicating that there is no unspecific binding of the fluorescent conjugated probe.

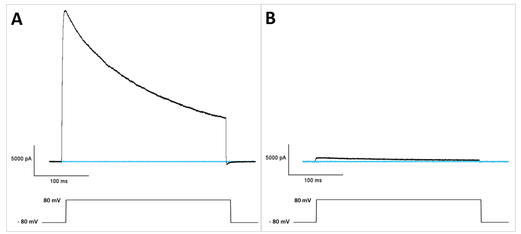

Fig. 2: Examples of current traces recorded from CHO-cells with high expression (A) and low expression (B) of the $K_V1.3$ channel, respectively, evoked by a depolarizing pulse to 80 mV from a 200 ms prepulse potential of –80 mV. The voltage protocol is shown in the bottom traces. A 10µM PAP-1 solution was used as blocker to confirm that the recorded currents were due to the $K_V1.3$ channel activity.

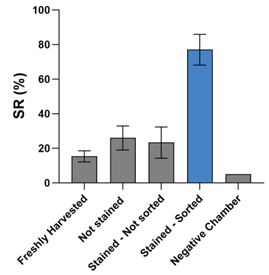

Fig. 3: Histogram showing the success rate of the QPatch experiments with, starting from left to right, freshly harvested cells, not stained cells that went through the staining steps, cells stained but not sorted, cells that were stained and sorted (blue bar), cells from the negative chamber of the sorting cartridge. n=3. Error bars ± SD.

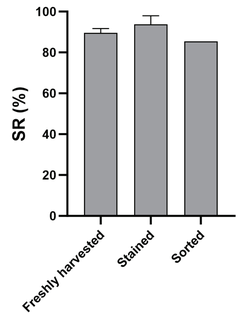

Fig. 4: Histogram showing the success rate of the QPatch tests without considering the current expression levels. This was done to evaluate the quality of the studied cells in terms of viability, capacitance, and resistance (i.e., applying only the filters for C_{slow} >4pF, and R_{mem} >200MΩ) in the case of, starting from left to right, freshly harvested cells, stained but not sorted cells, and cells that were stained and sorted. n=3. Error bars ± SD. From the histogram it is possible to conclude that the quality of the cells used for APC assays is not affected by the staining and sorting process.

Fig. 5: Decrease of QPatch tests success rate over time, compared to the success rate obtained with cells tested right after the sorting process.

Table 1: Examples of QPatch test results, showing the number of experiments started, the number of experiments completed, the number of experiments that passed the quality filters (I_{K_V} 1.3>400pA, C_{slow} >4pF, R_{mem} >200MΩ) and their ratio for each group, respectively. The average current level ($I_{KV1.3}$) and seal resistance (R_{mem}) ± their standard error of measurement (SEM) are also reported.

	Experiment count	Completed experiments	Experiment filter pass count	Experiment filter pass count/ completed experiments	Avg($I_{KV1.3}$) [nA] ± SEM	Avg(R_{mem}) [GΩ] ± SEM
Freshly harvested	45	44	6	0.14	16.8 ± 2.9	4.2 ± 0.4
Stained, not sorted	48	48	6	0.13	16.5 ± 3.2	13.7 ± 5.6
Not stained	46	45	8	0.18	16.9 ± 2.9	5.8 ± 0.7
Stained, sorted	45	43	36	0.84	19.9 ± 1.0	7.5 ± 2.1
Negative chamber	42	39	2	0.05	4.9 ± 0.2	2.4 ± 0.1

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