Detection of Ca++ Transients in iPSc-derived Cardiomyocytes: an HTS-ready Method of Measuring Cardiomyocyte Function

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Introduction

The detection of Ca++ transients in (recombinant) cell lines is widely used in HTS drug screening due to high specificity, robustness, throughput and sensitivity. Since calcium ions are the major trigger for the initiation of contraction in cardiomyocytes, such set-up can be used for HTS screening in early cardiac safety testing. Stem-cell-derived cardiomyocytes display a primary-like phenotype and a regular beating pattern, therefore being an ideal model to detect changes in calcium handling. The Hamamatsu FDSS kinetic plate readers are equipped with high-speed cameras, integrated dispenser heads and temperature control, allowing for detection of fast calcium signals under physiological conditions. We have used mouse stem-cell-derived Cor.At and human iPSc-derived Cor.4U cardiomyocytes in 96w and 384w plates to optimise assay conditions and to detect changes in calcium transients induced by cardiac ion channel modulators. Using iPSc-derived Cor.4U cardiomyocytes preincubated for 5 - 7 days in 384w plates, stable Ca++ transient signals could be measured over 45 min. The effect of several compounds on Ca++ transients in human iPSc-derived cardiomyocytes was detected using the Calcium 5 Assay Kit in the FDSS µCell.

Results

During assay optimisation, the dyes Fluo-4, Fluo-4FF, Fluo-8, and Fluo-8AM were tested. During a first measurement, half of a 384w plate was left untreated and served as assay control. P-rate, amplitude, and CTD90 were compared to the 100 msec mode. Human iPSc-derived Cor.4U cardiomyocytes were seeded at 10k per well in fibronectin-coated 96w Plates (Greiner µClear) and preincubated in standard Cor.4U Culture Medium for up to 10 days. Several Fluo dyes were tested in different buffer systems at either 2 µM or 2.5 µM with incubation at 37°C for 30 - 60 min. Measurements were performed at 37°C in the FDS7000EX equipped with a high sensitive CCD camera, and the high speed (8 msec) camera mode was compared to the 100 msec mode. Human iPSc-derived Cor.4U cardiomyocytes were seeded at 10k per well in fibronectin-coated 384w Plates (Greiner µClear) and preincubated in standard Cor.4U Culture Medium for 5 - 7 days prior measurement. Cells were loaded using the FLPR® Calcium 5 Assay Kit Component A (Molecular Devices, Sunnyvale, CA) dissolved in IMDM medium w/o FBS for 30 - 60 min at 37°C. Ca++ transients were measured at 37°C in the FDS7000EX equipped with a high sensitive CCD camera and the high speed (8 msec) camera mode was compared to the 100 msec mode.

Material and Methods

For assay optimisation, mouse stem-cell-derived Cor.At cardiomyocytes were thawed and seeded at 12k per well in fibronectin-coated 96w Plates (Greiner µClear) and preincubated in standard Cor.4U Culture Medium for up to 10 days. Several Fluo dyes were tested in different buffer systems at either 2 µM or 2.5 µM with incubation at 37°C for 30 - 60 min. Measurements were performed at 37°C in the FDS7000EX equipped with a high sensitive CCD camera, and the high speed (8 msec) camera mode was compared to the 100 msec mode. Human iPSc-derived Cor.4U cardiomyocytes were seeded at 10k per well in fibronectin-coated 384w Plates (Greiner µClear) and preincubated in standard Cor.4U Culture Medium for 5 - 7 days prior measurement. Cells were loaded using the FLPR® Calcium 5 Assay Kit Component A (Molecular Devices, Sunnyvale, CA) dissolved in IMDM medium w/o FBS for 30 - 60 min at 37°C. Ca++ transients were measured at 37°C in the FDS7000EX equipped with a high sensitive CCD camera and the high speed (8 msec) camera mode was compared to the 100 msec mode.

Table: Ca++ transient efficacy in a 384w plate

<table>
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<tr>
<th>Compound</th>
<th>Peak Number</th>
<th>RMP</th>
<th>AMP</th>
<th>PWD 90</th>
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<tr>
<td>B: Isradipine</td>
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<td>11,11</td>
<td>3,704</td>
<td>1,235</td>
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<td>C: TTX</td>
<td>50</td>
<td>16.7</td>
<td>3.53</td>
<td>1.92</td>
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</tr>
</tbody>
</table>

Fig. 1: Parameters measured and evaluated

Fig. 2: Characterisation of iPSc-derived cardiomyocytes

Fig. 3: Ca++-transients in iPSc-derived human Cor.4U cardiomyocytes

Fig. 4: Detection of compound effects on Ca++-transients in iPSc-derived human Cor.4U cardiomyocytes

Fig. 4 ctd: Detection of compound effects on Ca++-transients in iPSc-derived human Cor.4U cardiomyocytes

Fig. 4 ctd: Detection of compound effects on Ca++-transients in iPSc-derived human Cor.4U cardiomyocytes

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